The Kentucky Macroinvertebrate Bioassessment Index

Derivation of Regional Narrative Ratings for Assessing Wadeable and Headwater Streams

Kentucky Department for Environmental Protection
Division of Water
Water Quality Branch

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The Kentucky Macroinvertebrate Bioassessment Index

Derivation of Regional Narrative Ratings for Assessing Wadeable and Headwater Streams

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1.0 Introduction

Determining the ecological health of streams is the primary focus of the various aquatic monitoring programs in the Kentucky Division of Water (KDOW). Authority for KDOW's environmental programs comes from the federal Clean Water Act (CWA), Kentucky statute, and federal and state regulations. The monitoring integrates the collection of physical, chemical and biological elements to assess the quality of the aquatic environment. Monitoring tools such as biological indices must be developed for assessing stream condition to comply with provisions of the CWA. The KDOW uses combinations of algal, macroinvertebrate and fish community structure as indicators of waterbody health (KDOW 2002). Since the early 1900s, aquatic organisms have been used extensively in water quality monitoring and impact assessment (see review by Cairns and Pratt 1993), and macroinvertebrate assemblages have proven to be useful in detecting even subtle changes in habitat and water quality. To accurately characterize patterns of stream degradation, impact assessment procedures must be based on sound ecological principles and the ability to feasibly measure the response of a macroinvertebrate community to disturbance.

The purpose of this report is first to document the development of a statewide aggregate index for macroinvertebrates by identifying measurable biological attributes, or metrics, that can distinguish between reference and non-reference communities across regional scales. These attributes are then combined into an index of biotic integrity, or a Macroinvertebrate Bioassessment Index (MBI), based on a multimetric approach (Karr et al. 1986, Gerritsen 1995, Barbour et al. 1999). Second, this report defines regional MBI criteria for stream assessment. The index then ranks the quality of stream reaches affected by regional point and nonpoint source stressors arising from municipalities, agriculture, mining, silviculture, residential and commercial development, or road and bridge construction. Third, it also identifies those high quality or "Exceptional Waters" deserving regulatory protection under Kentucky's anti-degradation rules (401 KAR 5: 030 Section 1). Programmatically, the uses for the MBI are applicable for all general assessment and compliance monitoring associated with the Water Quality Branch (WQB), the Watershed Management Branch (WMB) and the Kentucky Pollutant Discharge Elimination System (KPDES) Branch.

1.1 Reference Conditions

To address levels of impact to any given stream, a firm understanding of the inherent biological variability and natural potential of streams in a collective region is necessary. This is accomplished using a regional reference approach (Hughes 1995), which is based on the range of conditions found in a population of sites or streams with similar physical characteristics and minimal human impact. Many federal, state and tribal agencies have used ecoregions (Omernik 1987), or modifications thereof, as a convenient, stratified means to understand regional differences in biological potential among waterbodies within their jurisdiction. The objectives of the Reference Reach Program in the Division's WQB are to collect and summarize data from least-disturbed streams using a regional framework in order to develop appropriate criteria for bioassessment interpretation. This regional sampling design is more robust than site-specific control methods and facilitates assessment at various scales (Barbour 1997). Prior agency reports on fish (KDOW 1997), algal (KDOW 1998) and macroinvertebrate (KDOW 2000a) communities inhabiting Kentucky's reference reach streams helped to develop a framework for establishing reference conditions in selected parts of the state.

The reference condition collectively refers to the range of quantifiable ecological elements (i.e., chemistry, habitat and biology) that are found in natural environments. In many regions of

Kentucky, finding reference streams can be a difficult task, because no regions are entirely without areas of human disturbance. To select reference quality (i.e., minimally- or least-disturbed) streams, the WQB uses a combination of narrative and quantitative physical attributes shown in Table 1. Additional agency data were also reviewed (e.g., presence/absence of dischargers, confined animal feeding operations, mines, oil and gas development and land cover) to help select candidate reference reaches.

Table 1. Summary of physical criteria used in the Reference Reach selection process.

Category	Criterion
1) riparian zone condition*	well-developed providing some canopy over the stream; presence of adequate aquatic habitats in the form of root mats, coarse woody debris and other allochthonous material
2) bank stability*	at least moderately stable with only a few erodible areas within the sampling station
3) degree of sedimentation*	the substrate is 25 percent or less embedded by fine sediment
4) suspended material	the water is relatively free from suspended solids during base flow conditions
5) evidence of nutrient enrichment	the substrate is relatively free from extensive algal mats that could smother benthic habitats
6) conductivity	conductivity is not highly elevated above what naturally occurs (region-specific)
7) aquatic habitat availability*	there is $\geq$ 70 percent (or >50 percent for low gradient) mix of rubble, gravel, boulders, submerged logs, root mats, aquatic vegetation or other stable habitats available for aquatic organisms
8) presence or absence of trash in the stream	solid waste within the stream and on the streambank is rare or absent
9) evidence of new land-use activities in the watershed	the land use conditions are unchanged compared to most recent topographic maps or aerial photos
10) accessibility of the site for collection	accessible

* Scored using the RBP Habitat Assessment forms (Barbour et al. 1999).

The application of the reference condition involves its comparison to streams exposed to environmental stress using defined sampling methodology and assessment criteria. Impairment would be detected if indicator measurements (e.g., biological indices, habitat rating, nutrient concentrations) fall outside the range of threshold criteria established by the reference condition.

2.0 Geographic Setting

2.1 General Physiography

Kentucky is physically diverse with mountainous, rolling hill and relatively flat topography. Geologically, it is comprised largely by Pennsylvanian-aged sandstones, Mississippian-aged limestones, Ordovician-aged limestones, and Tertiary and Quaternary alluvium and loess. From a statewide perspective, these factors contribute to rich geomorphic and chemical attributes of terrestrial and aquatic habitats. Although Pleistocene events have had some influence on natural drainage patterns in Kentucky (see Burr and Warren 1986), only a small portion of northern Kentucky was muted by glaciation; therefore, geologic and soil development and most drainage patterns have evolved over a relatively long period of time. In limestone regions, extensive karst has developed, creating diverse groundwater networks with numerous sinking and spring-fed streams. Human settlement and anthropogenic modifications to the landscape have also influenced the physical setting of Kentucky's watersheds. A diverse suite of land-use types (e.g., agriculture, resource extraction, silviculture, industrial and urban development) occurs throughout the Commonwealth, each causing direct and indirect impacts to aquatic ecosystems.

2.2 Ecological, Biological and Drainage Regions

An important component to developing regional MBI criteria is to test various regional classification schemes that account for the natural environmental variability in streams. Streams are products of their watersheds and valleys (Hynes 1975) and are directly influenced by physical characteristics of the surrounding landscape. Regionalization is a convenient way for resource agencies to manage and protect environmental resources (Gallant et al. 1989). One means to account for the physical and biological variation among areas is by the delineation of ecological regions, or ecoregions. Ecoregion maps are derived from information on geology, topography, soils, vegetation and land-use. Level III ecoregions of the United States were originally defined by Omernik (1987) and later modified (U.S. EPA 2000). Kentucky has seven Level III ecoregions (Figure 1a) that include ecoregions 68 (Southwestern Appalachians), 69 (Central Appalachians), 70 (Western Allegheny Plateau), 71 (Interior Plateau), 72 (Interior River Valleys and Hills), 73 (Mississippi Alluvial Plain) and 74 (Mississippi Valley Loess Plain). Many states have published Level IV subecoregions, and recently Woods et al. (2002) have delineated 25 subecoregions within Kentucky. KDOW is currently in the process of collecting data within all of these subecoregions. Stream classification using the Level IV subecoregional scheme will not be considered further until more information can be gathered. General lithology, land use and vegetation of the seven Level III ecoregions are summarized in Table 2.

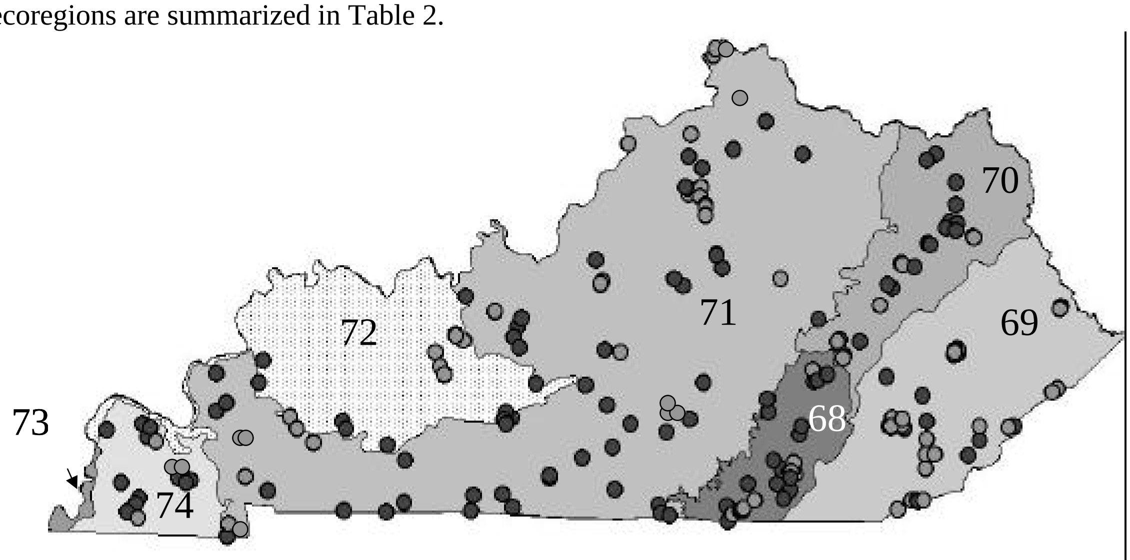

Figure 1a. Map of wadeable (dark circles) and headwater (light circles) reference sites distributed among Level III ecoregions. 68=Southwestern Appalachians, 69=Central Appalachians, 70=Western Allegheny Plateau, 71=Interior Plateau, 72=Interior River Valleys and Hills, 73, Mississippi Alluvial Plains, 74=Mississippi Valley Loess Plains.

Drainage basins have been known to influence aquatic faunal distributions, especially with fishes (Burr and Warren 1986) and mussels (Cicerello et al. 1991). KDOW recognizes 12 major river basins (Figure 1b) that include the Big Sandy, Upper Cumberland, Green, Kentucky, Licking, Little Sandy, Lower Cumberland, Mississippi (minor tributaries), Ohio (minor tributaries), Salt, Tennessee and Tradewater.

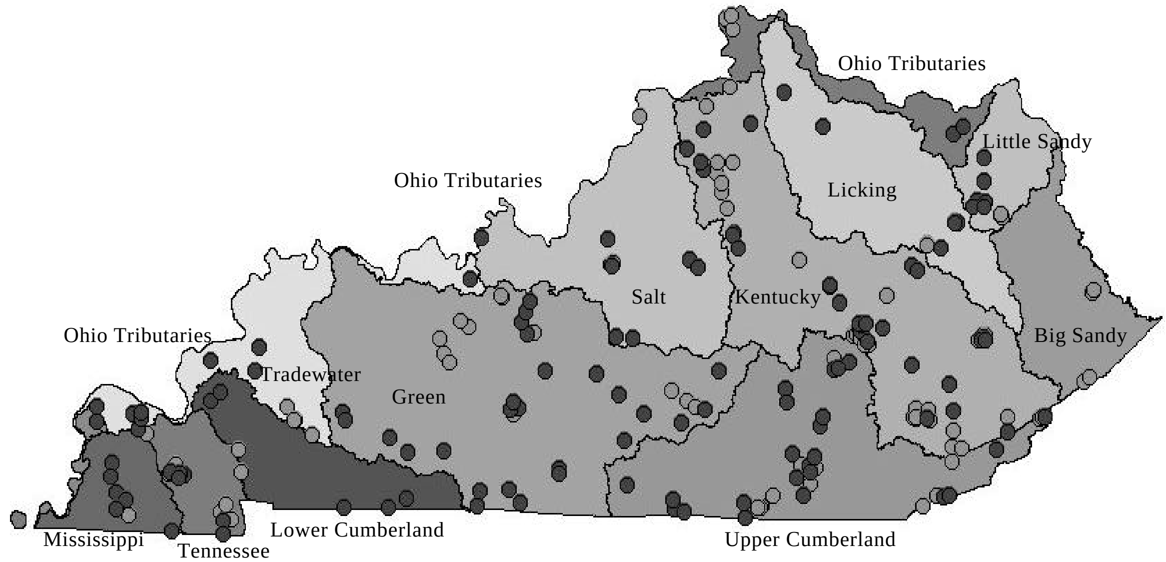

Figure 1b. Map of wadeable (dark circles) and headwater (light circles) reference sites distributed among major river basins.

Another regionalization scheme that KDOW has found helpful is to modify ecoregions *a posteriori* using biological data. Here, an analysis of the similarity among biological assemblages across geographic scales can help to simplify regional classifications of stream habitats for assessment purposes. Modified ecoregions, or bioregions, based on earlier KDOW studies (Pond et al. 2000, Pond and McMurray 2002, and KDOW unpub. data) are shown in Figure 1c. These regions correspond to generalized physiographic regions which include the Mountains (MT), Blue Grass (BG), Pennyroyal (PR) (includes Knobs-Norman Upland subecoregion 71c) and the combined Mississippi Valley/Interior River Lowland (MVIR).

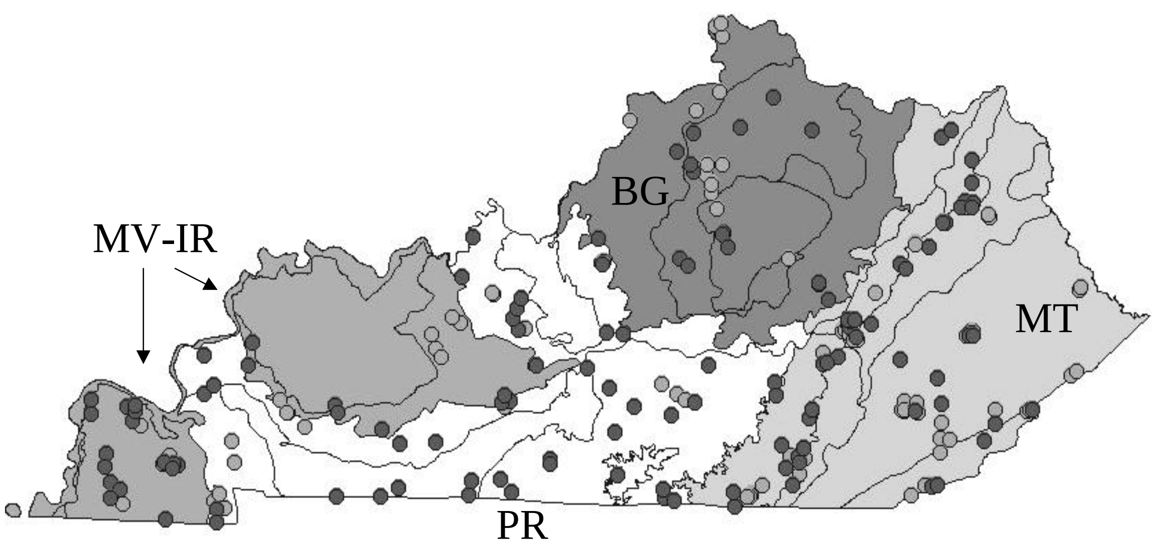

Figure 1c. Map of wadeable (dark circles) and headwater (light circles) reference sites distributed among bioregions. BG=Bluegrass, MT=Mountain, PR=Pennyroyal, MV-IR= Mississippi Valley-Interior River Lowlands. Solid lines mark Level IV subecoregion boundaries (see Woods et al. 2002).

Table 2. Generalized Level III Ecoregion attributes for Kentucky (taken from Woods et al. 2002).

Ecoregion	Landform/Geology	Potential Natural Vegetation	Land Use
Southwestern Appalachians (68)	Mixture of open, low mountains with a deeply-incised escarpment occuring in the west near the boundary with the Interior Plateau (71). The landscape is underlain by Pennsylvanian and Mississippian rock strata.	Mixed mesophytic forest generally restricted to the deeper ravines and escarpment slopes; mixed oaks with shortleaf pine dominate the upland forests.	Silviculture, mining, oil and gas drilling, agriculture, residential.
Central Appalachians (69)	High, dissected and rugged plateau made up of sandstone, shale, conglomerate and coal of Pennsylvanian age. Highest relief and elevation in state.	Mixed mesophytic forest but mixed oak forests common on drier sites including upper slopes and south-facing middle and lower slopes.	Silviculture, mining, oil and gas drilling, light agriculture, residential.
Western Allegheny Plateau (70)	Horizontally-bedded, Pennsylvanian sedimentary rock containing sandstone, siltstone, shales and coal. Some areas have eroded down to limestone and may have localized karst development.	Mixed mesophytic forest but mixed oak forests common on drier sites including upper slopes and south-facing middle and lower slopes.	Silviculture, mining, oil and gas drilling, moderate agriculture, residential.
Interior Plateau (71)	Irregular plains, open hills, knobs and large areas of karst topography. Underlain by Mississippian through Ordovician-age limestone, chert, sandstone, siltstone and shale.	Oak-hickory forest and bluestem prairie. Western mixed mesophytic forest on mesic slopes.	Cropland and pasture, silviculture, oil and gas drilling, urban development.
Interior River Valleys and Hills (72)	Undulating lowland was formed in non-resistant, non-calcareous sedimentary rock and coal of Pennsylvanian age. Large upland areas veneered by windblown material. Many wide, flat-bottomed, terraced valleys occur and are filled with alluvium, loess and lacustrine deposits.	Bottomland hardwood forests and swamp forests on poorly drained, nearly level sites; oak-hickory forests on upland areas.	Cropland and pasture, silviculture, coal mining, oil and gas drilling, urban development.
Mississippi Valley Alluvial Plain (73)	Rock stratum is almost exclusively composed of alluvial deposits. Mostly flat, broad floodplains with river terraces and levees provide the main elements of relief.	Southern floodplain forest and includes mixed deciduous bottomland forest dominated by water-tolerant oaks and maples and swamp forests of tupelo and bald cypress. Natural grasslands occupied sandy areas.	Cropland and pasture and residential.
Mississippi Valley Loess Plain (74)	Irregular plains, gently rolling hills and near the Mississippi River, bluffs. Mostly covered by thick loess and alluvium and underlain by Cretaceous and Tertiary coastal plain sediments.	Oak-hickory forest and a mosaic of bluestem prairie and oak-hickory forest. Low areas with cypress swamps and floodplain forests.	Cropland and pasture, silviculture, gravel mining, residential.

2.3 Stream Size

For macroinvertebrates, KDOW considers headwater (generally <5 mi^2), wadeable (5 to 200 mi^2), wadeable large river (>200 mi^2) and non-wadeable large river (>200 mi^2) separately in assessment criteria. Headwater and wadeable streams are discussed herein, while reference data collection for large rivers is currently under development. While these drainage area cutoffs are somewhat arbitrary, they are derived by careful observations and analysis of KDOW data. Hence, for MBI development and application, streams are categorized *a priori* by stream size (headwater or wadeable).

Headwater streams serve multiple functions (e.g., water supply, waste assimilation, flood control and ecological values) often overlooked in environmental planning and land-use decision making. These often-intermittent waterbodies are primarily 1st and 2nd, and few 3rd order streams that serve as the key interface between the surrounding landscape and larger waterbodies and provide goods and services in the form of high-quality water for downstream uses (Yoder et al. 2000, Wallace and Meyer 2001). In general, natural headwater streams in Kentucky are narrow, shallow, cool, heavily shaded, low in nutrients and dissolved ions, and biological diversity may be limited by reduced flow permanence. They are predominately heterotrophic, where energy is derived from allochthonous organic material provided by riparian vegetation (e.g., leaves, sticks and large woody debris). For bioassessment purposes, headwater streams are sampled in the spring index period (February through May). This period is when macroinvertebrates are the most diverse and abundant in these systems, thereby providing investigators with the maximum amount of information for assessment purposes. Furthermore, these streams are most likely to cease flow or dry up between the summer and fall seasons, and many obligate headwater taxa will be inactive or absent (KDOW unpub. data).

Wadeable streams (~5 to 200 mi^2) are perennial waterbodies generally ranging between 3rd and 5th order. They characteristically are wider, deeper, warmer and higher in solute concentrations than headwater streams. Wadeable streams in Kentucky also support some of the most productive and diverse fish communities (KDOW unpub. data). Likewise, macroinvertebrate communities inhabiting streams in this size category are considered the most diverse and productive along the stream continuum (Vannote et al. 1980). These larger waterbodies are predominately autotrophic, deriving most of their energy photosynthetically via algal and macrophyte communities. For bioassessment purposes, wadeable streams are sampled in the summer index period (June through September), generally corresponding to periods of normal flow when (1) sampling conditions are amenable and (2) macroinvertebrates are diverse and abundant.


Example headwater and wadeable stream reaches. Shown are UT Kentucky River (0.65 mi^2) and Kinniconick Creek (88 mi^2).

3.0 Sampling Methods

3.1 Database

All biological, habitat and chemical data used in these analyses are stored in KDOW's Ecological Data Application System (EDAS, v. 3.01) database. A total of 106 wadeable reference sites and 92 headwater reference sites were used to establish regional (e.g., ecoregions, bioregions or drainage basins) reference conditions for macroinvertebrates. These data were collected over a 5-year period between 1998 and 2003. Non-reference site data were collected through various other KDOW monitoring efforts including the intensive survey, watershed, ambient, nonpoint source and probabilistic monitoring programs. Data from combined non-reference sites (382 wadeable and 65 headwater) were collected over a 15-year period, with the majority of the events occurring between 1998 and 2002. All wadeable sites used in analysis were collected between June and September (summer index period). Headwater streams were sampled between mid-February and late-May (spring index period). Many sample events in the database that fell outside of these index periods were omitted from the analyses. Revisit or duplicate sampling was conducted at 15 reference sites to test repeatability of methods and variability of index scores.

3.2 Sampling Protocol

Macroinvertebrate sampling was conducted in accordance with *Methods for Assessing Biological Integrity of Surface Waters in Kentucky* (KDOW 2002). Stream sites were typically assessed at the reach scale, generally 100 m in length. For **wadeable** and **headwater** moderate/high gradient streams, a summary of sampling techniques is shown in Table 3 and Table 4, respectively (modified after Lenat 1988). *Quantitative* composited riffle samples (1 m^2 kicknet, 600 µm mesh) were analyzed separately from *qualitative* composited multi-habitat samples. Sample events collected with alternative methods (traveling kick method/multihabitat, surber sampler/multihabitat and combined kicknet/mutihabitat samples) were gleaned from the database and retained for analysis if (1) the number of individuals in a sample was greater than 300 and (2) best judgement indicated a relatively comparable collection to the methods shown in Tables 3 and 4. For each sample, an effort was made to rinse, inspect, and discard leaves and sticks, and sieve fine sediments so that 1 pint or less of material remained for each of the riffle and multihabitat samples. Each sample was then preserved in 95% ethyl alcohol.

Table 3. Summary of sampling methods for wadeable, moderate/high gradient streams.

Technique	Sampling Device	Habitat	Replicates (composited)
1m^2 Kicknet* (quantitative)	Kick Seine/Mesh Bucket	Riffle	4- 0.25m^2
Sweep Sample (multi-habitat)	Dipnet/Mesh Bucket	All Applicable	
Undercut Banks/Roots	"	"	3
Emergent Vegetation	"	"	3
Bedrock/Slabrock	"	"	3
Justicia beds	"	"	3
Leaf Packs	Dipnet/Mesh Bucket	Riffle-Run-Pool	3
Silt, Sand, Fine Gravel		Margins	
Coarse Sieve	US No. 10 Sieve		3
Rock Pick	Forceps/Mesh Bucket	Riffle-Run-Pool	15 rocks (5-5-5)
Wood Sample	Mesh Bucket	Riffle-Run-Pool	3-6 linear m

*Sample contents kept separate from other habitats

Table 4. Summary of sampling methods for headwater, moderate/high gradient streams.

Technique	Sampling Device	Habitat	Replicates (composited)
$1m^2$ Kicknet* (quantitative)	Kick Seine/Mesh Bucket	Riffle	4-$0.25m^2$
Sweep Sample (multi-habitat)	Dipnet/Mesh Bucket	All Applicable	
Undercut Banks/Roots	Dipnet/Mesh Bucket		3
Sticks/Wood			3
Leaf Packs	Dipnet/Mesh Bucket	Riffle-Run-Pool	3
Silt, Sand, Fine Gravel	Dipnet/Mesh Bucket	Margins	3
Rock Pick	Forceps/Mesh Bucket	Pool	5 boulders
Wood Sample	Forceps/Mesh Bucket	Riffle-Run-Pool	2 linear m

* Sample contents kept separate from other habitats

Low gradient streams are sampled differently than moderate/high gradient streams. These streams usually do not have naturally occurring riffles or other swift current habitat and are located predominately in ecoregions 72, 73 and 74. However, in headwater streams in these regions, shifty gravel riffles occur occasionally. Reaches of larger streams and rivers in other Kentucky ecoregions may also lack riffle/run habitats. The most productive habitats of these streams are typically woody snags, undercut banks and root mats, and aquatic vegetation. The sampling method follows, in part, the Mid-Atlantic Coastal Plain Streams Workgroup (MACS) protocol (MACS 1996), which is also described in Barbour et al. (1999). Essentially, the technique is considered "proportional sampling" where some predetermined number of sample units (20 in this case) is allocated among the distinct and productive meso-habitats in relation to their proportion found within a 100 m stream reach.

A sample unit is called a "jab" in which a D- or A-frame net is thrust into the targeted habitat in a jabbing motion for approximately 0.5 m and then swept with the net two or three times to collect the dislodged organisms. For example, in a 100 m stream reach, if woody snags made up roughly 50% of the reach, submerged root mats 25% and submerged macrophytes 25%, then ten jabs were allocated to the snags, five jabs allocated to the root mats and the last five jabs were allocated to the macrophytes. If a jab became heavily clogged with debris and sediment, the contents were discarded and the jab repeated. *All material was composited* into a wash bucket for further processing. Large leaves and twigs were washed, inspected and discarded to reduce the volume of the debris in the sample. Sand and sediment were elutriated using a bucket and 600 µm sieve. This was done until one pint or less of material remained, which was then preserved in 95% ethyl alcohol.

In the laboratory, all invertebrates were picked, identified to the lowest practicable taxon (usually genus/species) and enumerated. Proportional subsampling (25% or 50%) was done with quantitative riffle samples if they were estimated to contain more than 1,000 individuals. Here, a target number of 300 or more individuals in a 25% subsample was preferred. Afterward, the remaining sample was scanned for additional taxa under low magnification microscopy. Newly encountered taxa were added only for richness purposes. Counts of individual taxa in the subsample were multiplied by a factor dependent upon the proportion identified, so that an idea of total abundance could be realized. This procedure was done for less than 5% of samples used in this study, usually at streams in the more productive Interior Plateau ecoregion. This method of subsampling has been shown to be highly comparable to total sample counts and reduces time and effort when dealing with extremely high abundances (KDOW unpub. data).

Environmental parameter collection at monitoring sites included a combination of field parameters (dissolved oxygen, pH, conductivity and temperature) and habitat evaluation. Additional water chemistry sampling (e.g., nutrients, metals) was only conducted at less than half of the monitoring sites used in this study. Habitat features were scored with the EPA Rapid Bioassessment Protocol (RBP) Habitat Assessment procedure following Barbour et al. (1999). This procedure evaluates important habitat components such as epifaunal substrate quantity and quality, embeddedness, velocity/depth regimes, sediment deposition, channel flow status and channel alteration, stream bank stability, bank vegetative protection and riparian zone width. In low gradient streams, alternate parameters including pool substrate character, pool variability and channel sinuosity are substituted for embeddedness, velocity/depth regime and frequency of riffles, respectively.

Typical reference reaches in high-moderate gradient streams.

Typical reference reaches in low gradient streams.

4.0 Data Analysis

Data were analyzed to evaluate several objectives including stream classification, metric selection and testing, and index development and testing. These methods followed similar frameworks offered by Van Sickle (1997) for classification, Barbour et al. (1996,1999) and Gerritsen et al (2000a) for metric and index development, and Miltner and Rankin (1998) for index and metric testing with environmental stressors.

4.1 Community Classification

For bioassessment purposes, macroinvertebrates were classified into both regional and stream size categories. Regional classification schemes (e.g., ecoregions, basins and bioregions) are often used to compare areas of streams having biological similarity conforming to geographical orientation. Stream size also contributes to variability in macroinvertebrate communities by influencing abiotic factors such as temperature and flow regimes, substrate size distribution, habitat diversity and overall production (Vannote et al. 1980). In addition, land-use often changes predictably along the stream size continuum, indirectly affecting abiotic factors within aquatic systems.

4.1.1 Stream Size

KDOW has realized that there are inherent differences in macroinvertebrate community structure and thus, biological potential among smaller, headwater streams versus larger streams and small rivers in Kentucky. By separating these classes *a priori*, our intent was to reduce assessment error related to these natural differences. To verify the *a priori* designation of headwater and wadeable classes (see Section 2.3) we checked for colinearity of reference MBI values and drainage area. The appropriateness of *a priori* stream size designations (headwater or wadeable) for use with the MBI was examined with simple linear regression using $\log_{10}$ drainage area and MBI scores. Here, low r^2-values and nonsignificant (p>0.05) relationships would demonstrate that within individual classes, drainage area does not contribute to MBI score variability.

4.1.2 Regional Classification

Multivariate analyses were used to identify the best regional classification scheme (e.g., ecoregions, bioregions, basins) to be used in assessments with the MBI. A commonly used method for testing strengths of various classifications is mean similarity analysis (MEANSIM Version 6.0 (1998), Van Sickle 1997). This technique calculates the mean similarity of sites *within* classes ($\overline{W}$), and the mean similarity of sites *between* classes ($\overline{B}$) where the difference ($\overline{W} - \overline{B}$) is the classification strength (CS), or % of similarity that is explained by the classification. Statistical significance of the classification is accomplished by running a recommended 10,000 randomized permutations, or reassignments of the data (Van Sickle 1997). This process verifies if there is significant class structure compared to random assignments of the sites. The Bray-Curtis dissimilarity coefficient (inverted to similarity) using log abundance of invertebrate genera was used for the mean similarity analysis.

Another way to visualize classification is with ordination. Ordination is a graphical technique that compares community composition at sites in a spatial array that is based on either similarity/dissimilarity coefficients or eigenanalysis (Ludwig and Reynolds 1988). To verify classification strength, ordinations of regional classifications were constructed using non-metric multidimensional scaling (NMDS, Ludwig and Reynolds 1988) in conjunction with the Bray-Curtis coefficient (PC-ORD for Windows, MjM Software, Gleneden Beach, OR). For these analyses, genus-level resolution was used to reduce the statistical variability sometimes inherent in species-level data (Maxted et al. 2000). In general, NMDS attempts to arrange objects or communities found at individual sites in a spatial orientation with a particular number of dimensions so as to reproduce the observed statistical distances (Barbour et al. 1996). Sites that are taxonomically very similar will group closest to one another while sites that are the most dissimilar will be positioned farthest away in a two-dimensional ordination plot.

4.2 Metric Selection

A total of 33 biological attributes, or metrics (Table 5), was analyzed in previous studies (KDOW 1999 [Interior Plateau Ecoregion], Pond and McMurray 2002 [Eastern Coalfield Region]) for various qualities so that when combined into a single aggregate index, these metrics would be powerful at distinguishing site conditions. These metrics have also been described and evaluated in other federal and state programs (Plafkin et al. 1989, Resh and Jackson 1993, Kerans and Karr 1994, Deshon 1995, Barbour et al. 1999, Karr and Chu 1999, Arnwine and Denton 2000, and Gerritsen et al. 2000a). In some of these studies, a subset of metrics was selected by choosing those with high sensitivity, minimal redundancy and low variability.

After consideration of prior KDOW metric analyses, the present study documents the performance of seven core metrics. Metrics chosen as best candidates for the MBI are described below. For the present study, richness metrics were calculated from both quantitative and qualitative collections combined, whereas all other metrics were calculated using the quantitative riffle samples. For low-gradient streams sampled using the 20-jab composite method, metric values were calculated based on the total collection.

Genus Taxa Richness (TR). This refers to the total number of genera (semi-quantitative and qualitative samples combined) present in the composited sample. Taxa that cannot confidently be identified to the genus level (e.g., flatworms, mites, immatures of particular taxa, pupae, etc.) are recorded at the family level but still counted at the genus level as long as no other representatives of the group are encountered. In general, increasing taxa richness reflects increasing water quality, habitat diversity or habitat suitability.

Genus Ephemeroptera, Plecoptera, Trichoptera Richness (EPT). This is the total number of distinct genera (both semi-quantitative and qualitative samples combined) within the generally pollution-sensitive insect orders of Ephemeroptera, Plecoptera and Trichoptera found in the composited sample. Taxa that cannot confidently be identified to the genus level (e.g., early instars of particular taxa) are recorded at the family level but still counted at the genus level as long as no other representatives of the group are encountered. This metric will generally increase with increasing water quality, habitat diversity or habitat suitability.

Modified Hilsenhoff Biotic Index (mHBI). This metric requires species-level identification where possible. The HBI was developed to assess organic enrichment by summarizing the overall pollution tolerance of a benthic arthropod community with a single value (Klemm et al. 1990). Hilsenhoff (1988) developed tolerance values for a variety of macroinvertebrates from Wisconsin, and Plafkin et al. (1989) added additional tolerance values. However, KDOW uses tolerance values developed by the North Carolina Division of Environmental Management (NCDEM 2001) as well as values derived from KDOW data. These tolerance values have been regionally modified for streams of the southeastern United States. Several states, including Kentucky, have used the mHBI to assess impacts other than organic enrichment and found the mHBI to be a valuable metric. An increasing mHBI value indicates decreasing water quality.

The formula for Kentucky's mHBI is as follows:

$$m\,HBI = \frac{\sum n_i \, x \, a_i}{N}$$

where:
n_i = number of individuals within a species **(maximum of 25)**,
a_i = tolerance value of the species,
N = total number of organisms in the sample (**adjusted for** n_i **> 25)**

Modified Percent EPT Abundance (m%EPT). This metric measures the abundance of the generally pollution-sensitive insect orders of Ephemeroptera, Plecoptera and Trichoptera. The relatively tolerant and ubiquitous caddisfly genus *Cheumatopsyche* is excluded from the calculation. This genus can become *hyper*-dominant (i.e., excessively dominant) in riffle habitats under nutrient or chemical stress. Increasing m%EPT values indicate increasing water quality and/or habitat conditions.

Percent Ephemeroptera (%Ephem). The relative abundance of mayflies is calculated to detect impacts of metals and high conductivity associated with mining and oil well impacts. Ephemeroptera abundance normally declines in the presence of brine and metal contamination, as well as increased conductivity from a variety of disturbances including coal mining and dissolved solids loading from wastewater treatment plants (KDOW unpub. data). This metric is used only in headwater stream assessment since those mayfly species indigenous to smaller streams appear most sensitive.

Percent Chironomidae+Oligochaeta (%Chir+%Olig). This metric measures the relative abundance of these generally pollution tolerant organisms. Increasing abundance of these groups suggests decreasing water quality conditions from a variety of sources including coal mining, municipal waste, agriculture and industrial effluents (KDOW unpub. data). This metric was recently adopted by Tennessee for use in a multi-metric index (Arnwine and Denton 2000).

Percent Primary Clingers (%Clingers). This habit metric measures the relative abundance of those organisms that need hard, silt-free substrates on which to "cling". This metric was also recently adopted by Tennessee for use in a multi-metric index (Arnwine and Denton 2000). Merritt and Cummins (1996) and Barbour et al. (1999) list habits for most insect genera. Habit information for non-insect taxa can be determined from Pennak (1989), Thorp and Covich (1991), and Barbour et al. (1999).   Increasing metric values indicate increasing substrate stability.

Table 5. Original candidate metrics, abbreviations and expected response to disturbance. Modified from KDOW (1999) and Pond and McMurray (2002).

METRIC	Abbreviation	Response
No. of Intolerant Taxa[1]	IntolTax	Decrease
No. of Clinger Taxa[2]	ClngTax	Decrease
Rel. Abun. of Clingers	%Clingers	Decrease
Modified Hilsenhoff Biotic Index[3]	mHBI	Increase
TotalTaxa Richness	TR	Decrease
No. of Plecoptera Taxa	PlecoTax	Decrease
No. of Trichoptera Taxa	TrichTax	Decrease
No. of Ephemeroptera Taxa	EphemTax	Decrease
No. of Ephemeroptera+Plecoptera+Trichoptera	EPT	Decrease
Rel. Abun. of Chironomidae	%Chiro	Increase
Rel. Abun. Of Chironomidae+Oligochaeta	%Chir+Olig	Increase
Rel. Abun. Of Ephemeroptera	%Ephem	Decrease
Rel. Abun. Of Tolerants[4]	%Toler	Increase
Proportion of 5 Dominant Taxa	$\%DOM_5$	Increase
Rel. Abun. Of Tanytarsini	%Tany	Decrease
Rel. Abun. Of Hydropsychidae	%Hydro	Increase
Rel. Abun. Of Scrapers[5]	%Scrapers	Decrease
Ratio of EPT/ Chironomidae+Oligochaeta	EPT/C+O	Decrease
Total Individuals	TotInd	Variable
Rel. Abun. Of EPT	%EPT	Decrease
Rel. Abun. Of EPT (minus *Cheumatopsyche*)	m%EPT	Decrease
Rel. Abun. Of Trichoptera	%Trich	Variable
Rel. Abun. Of Diptera	%Dip	Increase
No. of Chironomidae Taxa	ChiroTax	Increase
Rel. Abun. Of Plecoptera	%Pleco	Decrease
Rel. Abun. Of Oligochaeta	%Oligo	Increase
Rel. Abun. Of Collector-Gatherers[5]	%Cllct	Variable
Rel. Abun. Of Shredders[5]	%Shred	Decrease
Shannon Diversity	Diversity	Decrease
Rel. Abun. Filter Feeders[5]	%Filtr	Variable
Rel. Abun. Of Dominant Taxon	%1Dom	Decrease
Rel. Abun. Of Baetidae	%Baetid	Increase
No. of Diptera Taxa	DipTax	Variable

[1]Based on tolerance values <3.0
[2]Based on habit designations in Merritt and Cummins (1996)
[3]Based on tolerance values provided in Lenat (1993), Hilsenhoff (1988), and KDOW (unpub. data)
[4]Based on tolerance values >7.0
[5]Based on functional feeding group designations in Merritt and Cummins (1996)

4.3 Metric Testing

For the revised statewide MBI discussed herein, three methods of metric efficacy are presented: (1) box plots that show discriminatory power or sensitivity; (2) a correlation matrix of reference metric values to detect metric redundancy; and (3) correlation analysis and box plots of metric values graphed against nutrient and habitat stressors.

(1) **Discriminatory power,** or the ability of metrics to discriminate between reference and non-reference sites, was done by statistical box plot comparisons. For this analysis, metrics are assigned sensitivity scores of 3, 2, 1 or 0 depending on the degree of interquartile (25^{th} to 75^{th} percentile) and median overlap between the populations of reference and non-reference sites (Figure 2, modified after Barbour et al. [1996]). If there was no interquartile overlap, metrics were considered to have excellent sensitivity and assigned a score of "3". Where there was some degree of overlap but medians fell out of the interquartile ranges, metrics were scored a "2". Metrics whose values showed considerable interquartile overlap and one of the medians fell within the other's interquartile range scored a "1". When both medians and interquartile ranges overlapped, metrics were considered to have poor sensitivity and scored a "0".

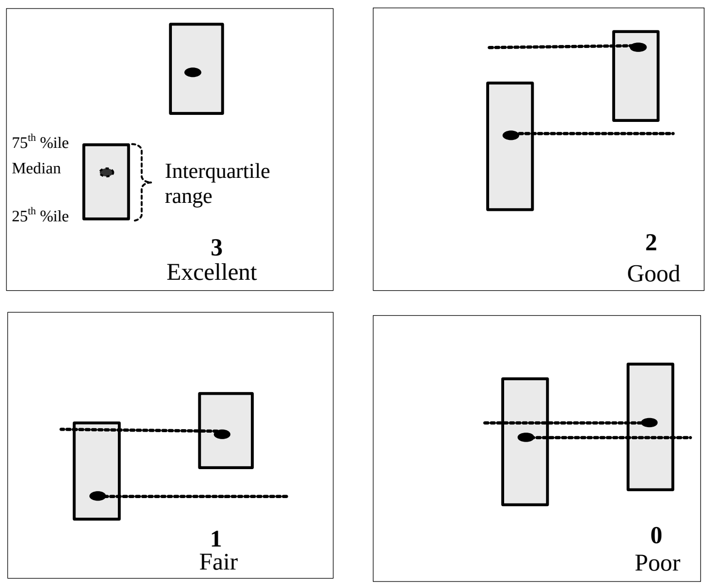

Figure 2. Hypothetical interquartile plots showing sensitivity or discriminatory power scoring criteria (after Barbour et al 1996).

(2) To detect **metric redundancy** (i.e., when two metrics provided the same information), a Pearson Correlation Analysis was run on reference metric values. Metric pairs that were highly correlated (r>0.75) were considered redundant, and inclusion of both metrics would provide no more information and perhaps compound assessment error. In this case, the weaker metric (e.g., lower discriminatory power, lower response to stressors) was omitted from further analysis.

(3) Metrics responding directly to **stressor gradients** are also valuable in an aggregate index (Karr and Chu 1999). To examine a nutrient concentration-metric response relationship, a data set of paired macroinvertebrate and nutrient samples (n=204) was evaluated. Metric values were correlated (Pearson's) with log transformed ammonia (NH_3), total Kjeldhal nitrogen (TKN), nitrate, total nitrogen (TN), total phosphorus (TP) and an interactive term (TN*TP). To allow for graphical interpretation of the response of various metrics with regard to the interaction of TN and TP concentrations, KDOW has adopted a categorical approach developed by Ohio EPA (Miltner and Rankin 1998). All of the nutrient data (i.e., statewide reference and non-reference) stored in EDAS were utilized to determine the 25^{th}, 50^{th}, 75^{th} and 90^{th} percentile distributions for TP (n=594) and TN (n=673) (Table 6). Bioassessment sites were placed into one of six categories (nutrient codes) based upon the percentile rankings for TP and TN at those sites. For example, a code rating of "1" was given to

sites having TP and TN concentrations less than the 25th percentile for both parameters. Sites were given a nutrient code rating of "2" if either TP or TN concentrations were less than the 50th percentile for either parameter. A category rating of "3" was given to sites having a TP concentration less than the 75th percentile and a TN concentration less than the 90th percentile. If a site had a TP concentration greater than the 75th percentile irrespective of TN, then the site was placed into category "4". Sites were given a category rating of "5" if both TP and TN concentrations were greater than the 90th percentile. Finally, if ammonia concentration (a toxic stressor) was greater than 1.0 mg/l, then the site was given a category rating of "6".

Table 6. Nutrient code designations for Total Nitrogen (TN) and Total Phosphorus (TP) (in mg/l) derived from dataset corresponding to all biological sample events (after Milton and Rankin 1998).

Code	Nutrient Interaction	Percentile	TP (n=594)	TN (n=673)
1	both $\leq TP_{25}$ TN_{25}	25th	0.014	0.386
2	either $\leq TP_{50}$ TN_{50}	50th	0.045	0.860
3	$\leq TP_{75}$, $<TN_{90}$	75th	0.163	1.763
4	$>TP_{75}$, $<>N_{90}$	90th	0.710	4.178
5	both $\geq TP_{90}$ TN_{90}			
6	$NH_3 \geq 1.0$ mg L^{-1}			

RBP habitat scores were also used to measure metric responsiveness to stress. A Spearman correlation analysis was run on habitat and biological metrics. While the correlation of biological metrics to total habitat score is informative, the WQB has recognized a subset of 7 of the 13 metrics (both high and low gradient) that more strongly drives community performance (epifaunal substrate, embeddedness, sediment deposition, velocity/depth regime, riparian zone width, pool variability and channel sinuosity). As with the nutrient gradient, a categorical approach was used with habitat parameters so that invertebrate metrics could be graphed with statistical box plots. A paired data set of macroinvertebrate collections and habitat evaluations was analyzed (n=353). Five categories based on the 75th, 50th, 25th and 10th percentiles of all habitat data stored in EDAS were used to assign habitat stress points (0 to 4) to each of the habitat parameters. Stress points were then summed for each sample event, and the site was assigned to one of five habitat stress categories (Table 7).

Table 7. Designation of site habitat stress codes using subset of RBP habitat parameters (a.) parameter percentile distributions, (b.) stress point scoring, (c.) stress code assignment.

a.

%ile	Embedded Score	Epifaunal Substrate	Sediment Deposition	Vel/Depth Regime	Riparian Zone	Pool Variability	Channel Sinuosity
75th	18	18	16	18	19	18	17
50th	16	16	13	16	15	16	13
25th	13	11	8	12	10	12	9
10th	8	7	6	9	5	9	6
n=	483	595	595	483	595	112	112

b.

Habitat Parameter %ile	Habitat Stress Points
> 75th	0
50 to 75th	1
25 to 50th	2
10 to 25th	3
<10th	4

c.

Range of Stress Points	Habitat Stress Code
0--4	1
5--9	2
10--14	3
15--19	4
20--24	5

4.4 Metric Scoring and Index Development

Metrics values were normalized by assigning scores so that they could be uniformly compared and aggregated into a multimetric index (Gerritsen 1995). Previous KDOW studies (KDOW 1999, Pond and McMurray 2002) used different scoring methods. The one adopted for the revised statewide MBI was the percent of standard method (Barbour et al. 1999, Gerritsen et al. 2000a, Pond and McMurray 2002), where each metric was calculated based on the range of metric values below the 95th %ile. This scoring method is also being currently used with diatom and fish community assessments at the KDOW (KDOW unpub. data). Here, metric values are standardized to the approximated "best" values found in the statewide reference dataset. The raw values of the positive disturbance response metrics (mHBI and %Chir+Olig) are first inverted to provide symmetry among all metrics. Each metric is then scored on a continual scale of 0-100 percent, and the MBI is calculated as the average of all equally weighted metric scores (after Gerritsen et al. 2000a). If a calculated metric scored over 100 (i.e., a value above the 95th %ile) then it was corrected to the maximum score of 100. The formulae for calculating metric scores are shown in Table 8. The final MBI score is the average of all individual metric scores (see section 5.4 for example calculations).

Table 8. Examples of metric scoring formulae for the Macroinvertebrate Bioassessment Index.

Metric	Formula
TR	$\frac{TR}{95th\%ile} X100$
EPT	$\frac{EPT}{95th\%ile} X100$
mHBI	$\frac{10-mHBI}{10-5th\%ile} X100$
m% EPT	$\frac{m\%EPT}{95th\%ile} X100$
%Ephem[1]	$\frac{\%Ephem}{95th\%ile} X100$
%Clingers	$\frac{\%Clingers}{95th\%ile} X100$
% Chir+Olig	$\frac{100-\%Chir+Olig}{100-5th\%ile} X100$

[1] %Ephem used only with headwater stream assessments.

4.5 MBI Narrative Ratings

To rate individual sites with MBI assessment scores, regional thresholds for both wadeable and headwater streams were established to assign narrative water-quality rankings of Excellent, Good, Fair, Poor and Very Poor. These rankings were based on percentile distributions of regional reference MBI scores. Although we did not test the utility of the "Very Poor" category, this rating recognizes or "flags" those most severely impaired streams that may require prioritization with regard to remedial actions. While the use of the 25th %ile of reference scores is often used to establish the biocriterion (Barbour et al. 1996, Barbour et al. 1999, Gerritson 1995), the WQB recognizes that there are varying levels of perceived reference site quality among regions and that alternative thresholds might be considered after review of the data (see Section 5.7). Sites rating as "Excellent" will be considered for listing as "Exceptional Waters" for antidegradation purposes (401 KAR 5:030 Section 1).

5.0 Results and Discussion

5.1 Regional Classification

The mean similarity analysis revealed that modified ecoregions, or bioregions, had the greatest classification strength in wadeable streams (14%) followed by Level III ecoregions (10%) and river basins (6.4%) (Table 9). In headwater streams, the same pattern was found with bioregions having the best classification efficiency (18%), followed by ecoregions (14%) and river basins (9.6%) (Table 10). Bioregional groupings were also demonstrated to be superior to ecoregions or catchments in classifying streams in Florida (Barbour et al. 1996), Wyoming (Gerritsen et al. 2000b) and Mid-Atlantic Coastal regions (Maxted et al. 2000). Moreover, Waite et al. (2000) found that there was little difference in macroinvertebrate communities among ecoregions in the Mid-Atlantic Highlands, a region that shares Level III ecoregions with Kentucky (i.e., Central Appalachians and Western Allegheny Plateau). Pond and McMurray (2002) found similar results in reference headwater streams scattered throughout eastern Kentucky's mountain ecoregions. This logic implies that although there might be discernible differences among ecoregions with regard to geology, topography, vegetation, etc., the distribution of stream macroinvertebrates may be more homogenous when combined within similar Kentucky ecoregions (e.g., mountain ecoregions, lowland ecoregions). In contrast to these ecoregion combinations, many Kentucky naturalists and Woods et al. (2002) have separated the Interior Plateau ecoregion into BG and PR bioregions because of geological, floral and fauna differences. Our results confirm that this separation has proved to be useful for macroinvertebrate communities.

Table 9. Mean within- ($\overline{W}$) and between- ($\overline{B}$) group similarity and classification strength (CS) for candidate classifications of wadeable reference streams.

	No. of Groups	$\overline{W}$	$\overline{B}$	$\overline{W}$ - $\overline{B}$ (CS)	*p*-value
Ecoregions	6*	0.384	0.285	0.10	<0.0001
Bioregions	4	0.415	0.274	0.14	<0.0001
Basins	12	0.362	0.298	0.064	<0.0001

*Ecoregion 73 omitted from analyses.

Table 10. Mean within- (*W*) and between- (*B*) group similarity, and classification strength (CS) for candidate classifications of headwater reference streams.

	No. of Groups	$\overline{W}$	$\overline{B}$	$\overline{W}$ - $\overline{B}$ (CS)	*p*-value
Ecoregions	6*	0.453	0.307	0.14	<0.0001
Bioregions	4	0.439	0.261	0.178	<0.0001
Basins	10	0.431	0.335	0.096	<0.0001

*Ecoregion 73 omitted from analyses.

Ordinations using NMDS confirmed that the bioregion classification scheme demonstrates good concordance (despite some overlap among BG, PR and MT sites) among region-specific macroinvertebrate communities in wadeable reference streams (Figure 3). Headwater streams also displayed good groupings consistent with bioregional representation (Figure 4). Although headwater stream classification strength was greater than wadeable sites in all three regional schemes, this may be an artifact of data distribution since the majority of sites were biased toward the MT bioregion (50%), which may have an effect on the mean similarity results. NMDS ordinations showed that for headwater reference sites in the PR and MVIR regions, groupings had more overlap compared to the wadeable site ordinations and that MVIR streams displayed the most variability overall. This contradiction may be remedied with future sampling in additional reference streams in the PR and MVIR. Until more data can be collected in Level IV ecoregions, the four-bioregional classification will be used for regional bioassessments (see map in Appendix A).

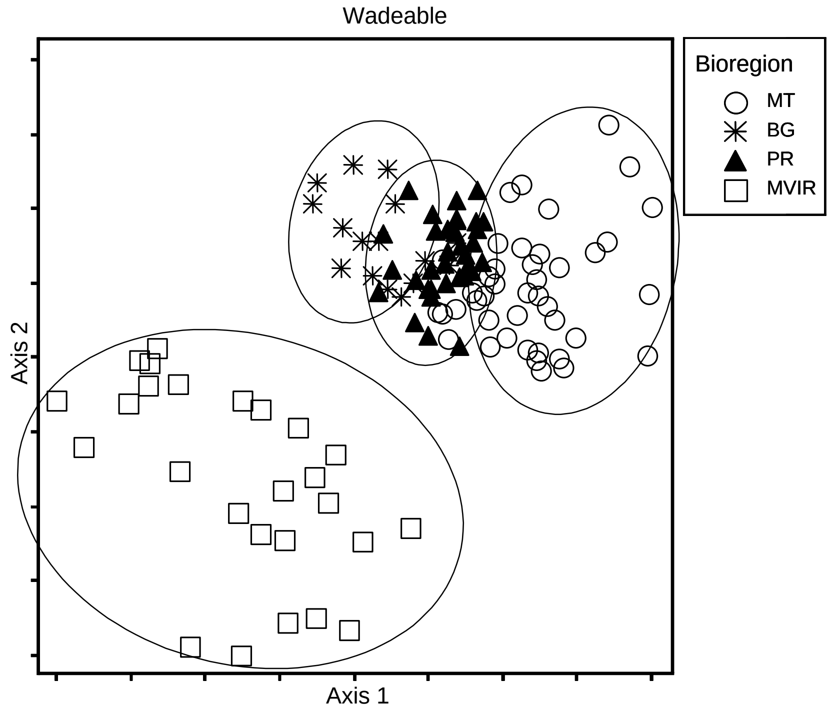

Figure 3. NMDS ordination of reference wadeable streams by bioregion. Ellipses drawn by eye to emphasize geographic separation. MT=Mountains, BG=Blue Grass, PR=Pennyroyal, MVIR=Mississippi Valley-Interior River.

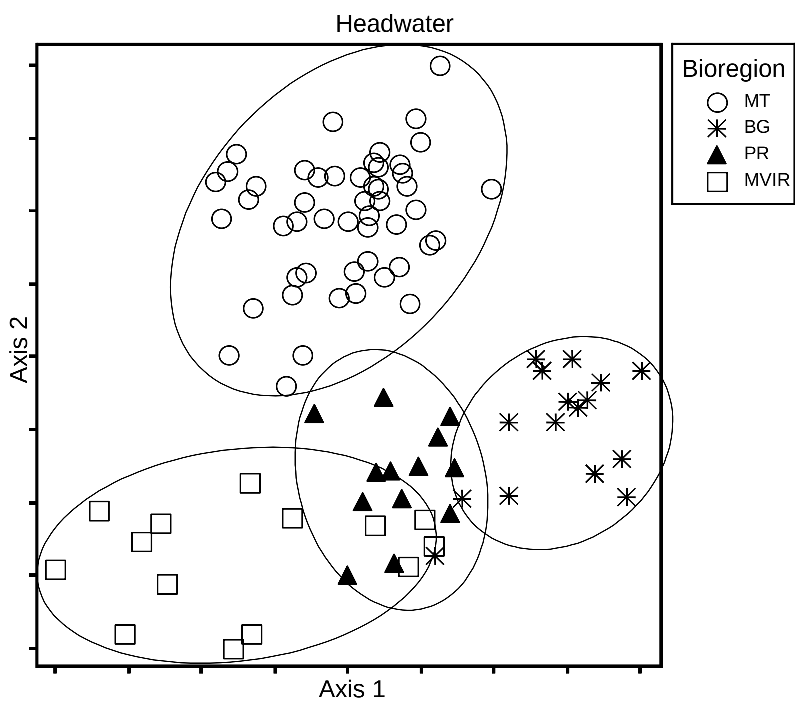

Figure 4. NMDS ordination of reference headwater streams by bioregion. Ellipses drawn by eye to emphasize geographic separation. MT=Mountains, BG=Blue Grass, PR=Pennyroyal, MVIR=Mississippi Valley-Interior River.

5.2 Macroinvertebrate Abundance and Composition

The reference site dataset consisted of 106 wadeable sites containing 286 genera and 92 headwater sites represented by 235 genera. The average abundance of organisms collected per sample event was 590 (±90, 95% C.I.) in wadeable streams and 697 (±176, 95% C.I.) in headwater streams. Extreme abundances (e.g., >2000/sample) were found in reference streams in the BG and PR (Interior Plateau ecoregion). Lowest abundances were more frequently found in the MVIR region. In general, taxa richness was variable in headwater and wadeable streams among bioregions (Figure 5a). MT and PR streams displayed the highest richness in both headwater and wadeable streams. BG and MVIR streams had the lowest richness values. EPT values were similar in that MT and PR streams yielded more taxa compared to BG and MVIR streams at both spatial scales (Figure 5b). Compared to other regions, MT headwater and wadeable EPT richness were highly similar. On average, MVIR streams yielded the lowest EPT richness expectations in Kentucky, a pattern likely related to habitat rather than water quality factors.

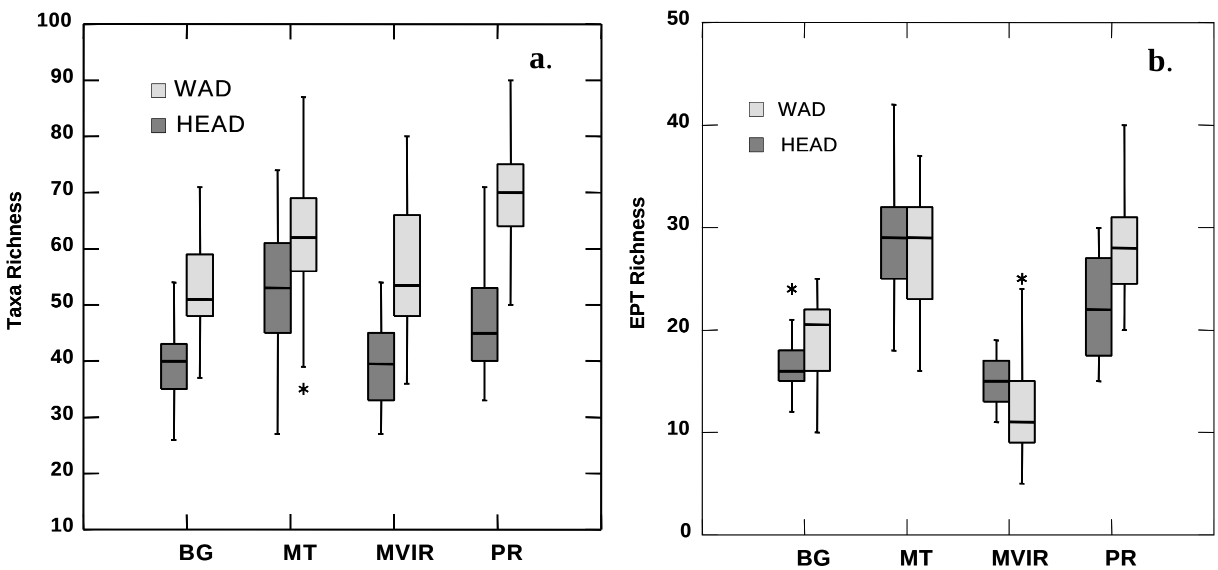

Figure 5. Box plots of species-level taxa richness (a) and EPT richness (b) for wadeable (WAD) and headwater (HEAD) reference sites by bioregion.

In terms of taxonomic composition, it is often informative to directly compare taxa that are both frequently and abundantly found at headwater and wadeable regional reference sites. Tables 11 and 12 show the top 15 genera found at wadeable and headwater bioregional reference sites, respectively, based on taxon mean relative abundances and relative frequency (mean relative abundance + relative frequency = importance). These taxa lists can be used as supplemental information and allow for interpretive taxonomic comparisons of the reference condition with data from new sites.

In wadeable streams, Ephemeroptera and Trichoptera generally dominated these lists, followed by elmid and psephenid beetles. Surprisingly, many of the most common taxa were shared among all bioregions despite the relatively strong separation revealed by the ordinations. This suggests that either there were large enough differences in individual taxon abundances among sites between bioregions, or less common species (i.e., those not listed as top 15) were more influential in the ordination and similarity analyses. Moreover, many of these taxa are regarded as facultative to stress rather than sensitive. For instance, the caddisfly genus *Cheumatopsyche* and the mayfly genus *Stenonema* were generally the most commonly encountered genera at reference sites in all bioregions, and several genera (e.g., *Nigronia*, *Baetis*, *Chimarra*, *Polypedilum*, *Psephenus* and *Thienemannimyia*) were common in three of the four bioregions.

In headwater streams, EPT taxa were the most frequently encountered. Stoneflies were more common in headwater streams than in wadeable sites. This observation is consistent with the fact that many stenothermic stonefly species are cool- or cold-water adapted and they are most diverse, abundant and active in the winter and spring months (Stewart and Stark 1988). We have observed that winter stoneflies such as capniids (e.g., *Allocapnia*) and taeniopterygids (e.g., *Taeniopteryx*) can become *hyper*-dominant in small streams in the late fall and winter. They were not numerically important in spring headwater communities in this study since these families are some of the first to emerge as adults, as early as February or March in Kentucky. The stonefly genera *Amphinemura* and *Isoperla* were in the top 15 taxa list in all bioregions; other genera (e.g., *Paraleptophlebia*, *Leuctra*, *Rhyacophila*, *Neophylax* and *Simulium*) were important in three of the four bioregions.

Table 11. Top 15 genera collected from reference **wadeable** streams by bioregion (mean relative abundance + relative frequency = relative importance).

BG (n=13)				**MT (n=44)**			
Genus	Rel. Abun.	Rel. Freq.	Imp	Genus	Rel. Abun.	Rel. Freq.	Imp
Stenelmis (3 spp.)	7.9	100.0	107.9	*Stenonema* (5 spp.)	9.3	97.3	106.6
Psephenus herricki	7.3	100.0	107.3	*Isonychia* sp.	10.7	89.2	99.9
Cheumatopsyche sp.	13.9	92.3	106.2	*Cheumatopsyche* sp.	7.5	91.9	99.4
Stenonema (4 spp.)	2.3	92.3	94.6	*Acroneuria* (3 spp.)	4.4	83.8	88.2
Lirceus fontinalis	2.0	92.3	94.3	*Optioservus* (2 spp.)	4.0	81.1	85.1
Orconectes (2 spp.)	1.2	92.3	93.5	*Nigronia* (2 spp.)	3.5	75.7	79.2
Baetis (3 spp.)	10.5	76.9	87.4	*Ceratopsyche* (3 spp.)	4.8	73.0	77.8
Perlesta spp.	3.8	76.9	80.7	*Baetis* (4 spp.)	2.6	67.6	70.2
Acroneuria (2 spp.)	1.7	76.9	78.6	*Leuctra* sp.	2.4	62.2	64.6
Nigronia (2 spp.)	1.4	76.9	78.3	*Polypedilum* (4 spp.)	2.2	62.2	64.4
Thienemannimyia gp.	1.0	76.9	77.9	*Psephenus herricki*	3.2	59.5	62.6
Sphaerium sp.	1.0	76.9	77.9	*Chimarra* (2 spp.)	5.5	56.8	62.2
Neoperla sp.	5.4	69.2	74.6	*Atherix* sp.	2.5	54.1	56.5
Polypedilum (4 spp.)	3.2	69.2	72.4	*Acentrella* (spp.)	1.1	51.4	52.5
Chimarra (2 spp.)	2.9	69.2	72.2	*Hydropsyche* (3 spp.)	1.7	43.2	44.9

PR (n=37)				**MVIR (n=24)**			
Genus	Rel. Abun.	Rel. Freq.	Imp	Genus	Rel. Abun.	Rel. Freq.	Imp
Cheumatopsyche sp.	11.6	97.1	108.7	*Physella* sp.	3.2	95.5	98.6
Stenonema (5 spp.)	9.9	91.2	101.1	*Dubiraphia* (2 spp.)	7.0	77.3	84.2
Isonychia sp.	12.3	88.2	100.5	*Cheumatopsyche* sp.	4.9	72.7	77.7
Stenelmis (3 spp.)	4.9	91.2	96.0	*Caenis* (4 spp.)	6.2	63.6	69.9
Baetis (3 spp.)	6.4	88.2	94.6	*Polypedilum* (5 spp.)	1.6	68.2	69.8
Elimia (3 spp.)	3.8	82.4	86.1	*Simulium* sp.	3.5	63.6	67.2
Psephenus herricki	3.1	79.4	82.5	*Lirceus fontinalis*	3.2	59.1	62.3
Nigronia (2 spp.)	2.0	79.4	81.4	*Stenonema* (4 spp.)	2.3	59.1	61.4
Corydalus cornutus	1.1	76.5	77.6	*Acerpenna* (2 spp.)	1.8	59.1	60.9
Polypedilum (4 spp.)	2.3	73.5	75.8	*Sialis* sp.	1.4	54.5	55.9
Hydropsyche (4 spp.)	1.5	67.6	69.1	*Thienemannimyia* gp.	1.3	54.5	55.8
Thienemannimyia gp.	0.9	64.7	65.6	*Ablabesmyia* (3 spp.)	1.1	54.5	55.6
Chimarra (2 spp.)	6.7	58.8	65.5	*Boyeria vinosa*	2.4	50.0	52.4
Rheotanytarsus sp.	0.8	61.8	62.6	*Enallagma* (3 spp.)	1.9	50.0	51.9
Optioservus (2 spp.)	5.0	55.9	60.9	*Chironomus* sp.	1.7	50.0	51.7

Table 12. Top 15 genera collected from reference **headwater** streams by bioregion (relative abundance + relative frequency = importance).

BG (n=17) Genus	Rel. Abun.	Rel. Freq.	Imp	**MT** (n=49) Genus	Rel. Abun.	Rel. Freq.	Imp
Isoperla sp.	19.5	94.1	113.6	*Ephemerella* (3 spp.)	13.8	95.2	109.0
Amphinemura (2 spp.)	11.6	100.0	111.6	*Epeorus* (2 spp.)	9.2	97.6	106.8
Lirceus fontinalis	11.3	94.1	105.5	*Ameletus* sp.	8.3	95.2	103.6
Acentrella (2 spp.)	5.4	100.0	105.4	*Amphinemura* (3 spp.)	7.8	95.2	103.1
Rhyacophila (3 spp.)	3.1	100.0	103.1	*Neophylax* sp.	2.0	97.6	99.7
Stenelmis (2 spp.)	1.9	82.4	84.2	*Leuctra* sp.	2.0	97.6	99.6
Unid. Planariid	0.5	82.4	82.9	*Rhyacophila* (8 spp.)	1.4	97.6	99.1
Neophylax sp.	1.8	76.5	78.3	*Cambarus* (7 spp.)	0.7	97.6	98.3
Thienemannimyia gp.	1.4	76.5	77.9	*Eurylophella* (3 spp.)	0.5	97.6	98.1
Simulium sp.	1.3	76.5	77.7	*Pycnopsyche* (3 spp.)	0.3	97.6	97.9
Ochrotrichia sp.	1.2	76.5	77.6	*Tipula* sp.	1.0	95.2	96.2
Eukiefferiella sp.	0.9	76.5	77.3	*Diplectrona modesta*	3.0	92.9	95.9
Eclipidrilus sp.	0.5	76.5	76.9	*Hexatoma* sp.	1.3	88.1	89.4
Ameletus sp.	2.3	70.6	72.9	*Isoperla* sp.	1.5	85.7	87.2
Paraleptophlebia sp.	1.8	70.6	72.4	*Acroneuria* (2 spp.)	1.2	85.7	86.9
Leuctra sp.	1.8	70.6	72.4				

PR (n=12) Genus	Rel. Abun.	Rel. Freq.	Imp	**MVIR** (n=14) Genus	Rel. Abun.	Rel. Freq.	Imp
Amphinemura (2 spp.)	9.5	100.0	109.5	*Paraleptophlebia* sp.	8.9	92.9	101.8
Leucrocuta sp.	15.9	90.9	106.8	*Amphinemura* sp.	6.6	92.9	99.5
Leuctra sp.	5.3	100.0	105.3	*Simulium* sp.	5.2	85.7	90.9
Paraleptophlebia sp.	5.2	100.0	105.2	*Plauditus* (2 spp.)	2.1	85.7	87.9
Rhyacophila (4 spp.)	2.7	100.0	102.7	*Thienemannimyia* gp.	0.9	85.7	86.6
Parametriocnemus sp.	1.0	100.0	101.0	*Perlesta* sp.	7.3	78.6	85.8
Tipula sp.	0.5	100.0	100.5	*Rhyacophila* (2 spp.)	0.8	78.6	79.4
Lirceus fontinalis	5.1	90.9	96.0	*Isoperla* sp.	5.6	71.4	77.0
Simulium sp.	2.5	90.9	93.4	*Caenis* (2 spp.)	2.8	71.4	74.3
Stenelmis (2 spp.)	2.2	90.9	93.1	*Centroptilum* sp.	1.6	71.4	73.1
Thienemannimyia gp	1.4	90.9	92.3	*Polypedilum* (3 spp.)	2.2	64.3	66.5
Stenonema (3 spp.)	1.3	90.9	92.2	*Caecidotea* sp.	2.2	64.3	66.4
Isoperla sp.	9.3	81.8	91.1	*Ironoquia* sp.	1.4	64.3	65.7
Helichus (2 spp.)	0.4	81.8	82.2	*Helichus* (2 spp.)	1.3	64.3	65.6
Neophylax sp.	0.3	81.8	82.1	*Leucrocuta* sp.	7.7	57.1	64.9

5.3 Metric Selection and Testing

For the discriminatory power test in wadeable and headwater streams (Figures 6 and 7, respectively), box plots of G-TR, G-EPT, mHBI, m%EPT, %Ephem (headwater sites only), %Chir+Olig and %Clingers among reference and non-reference sites showed good to excellent sensitivity (score of 2 or 3) among most or all bioregions. In wadeable streams, the %Clinger metric showed high bioregional variability ranging from poor sensitivity (score of 0) in the limestone regions (BG and PR) to good and excellent in the MT and MVIR regions, respectively. The metric showed better discrimination among headwater sites, but scored poor in the PR region. The Chir+Olig metric showed only fair discriminatory power in the MVIR wadeable sites but good sensitivity in other bioregions. Sensitivity of this metric was slightly better in headwater streams, but it had only fair discriminatory power in the BG. Despite those cases where metrics scored a 0, reference medians were always higher than non-reference medians. Metric values for all reference sites by bioregion are listed in Appendices B through E.

Wadeable

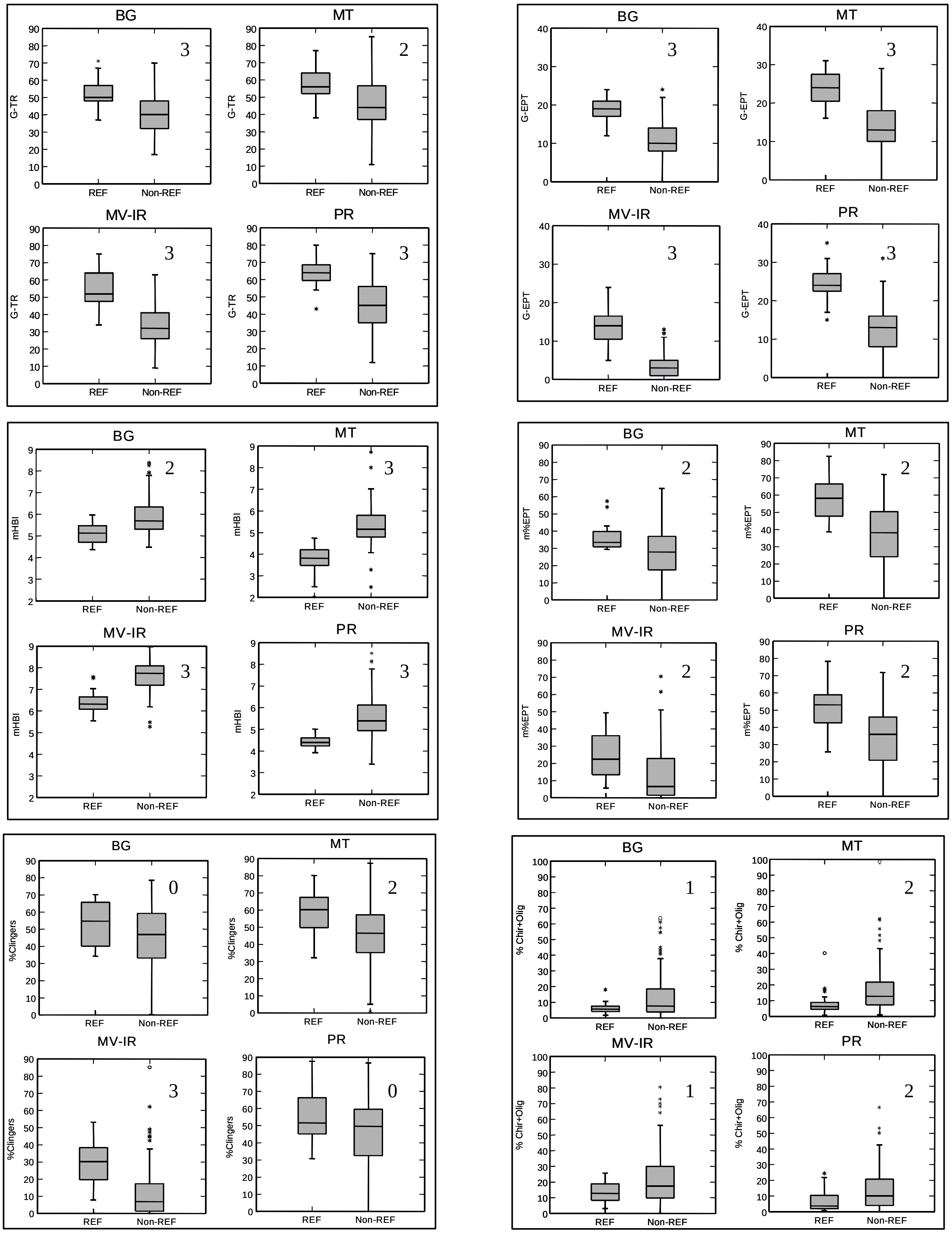

Figure 6. Box plots showing discriminatory power for Genus Taxa Richness (G-TR), Genus EPT (G-EPT), mHBI, m%EPT, %Clingers and %Chir+Olig in wadeable reference (REF) and non-reference (Non-REF) streams. Scores in the right upper right-hand location of each plot correspond to sensitivity scores (see Figure 2).

Headwater

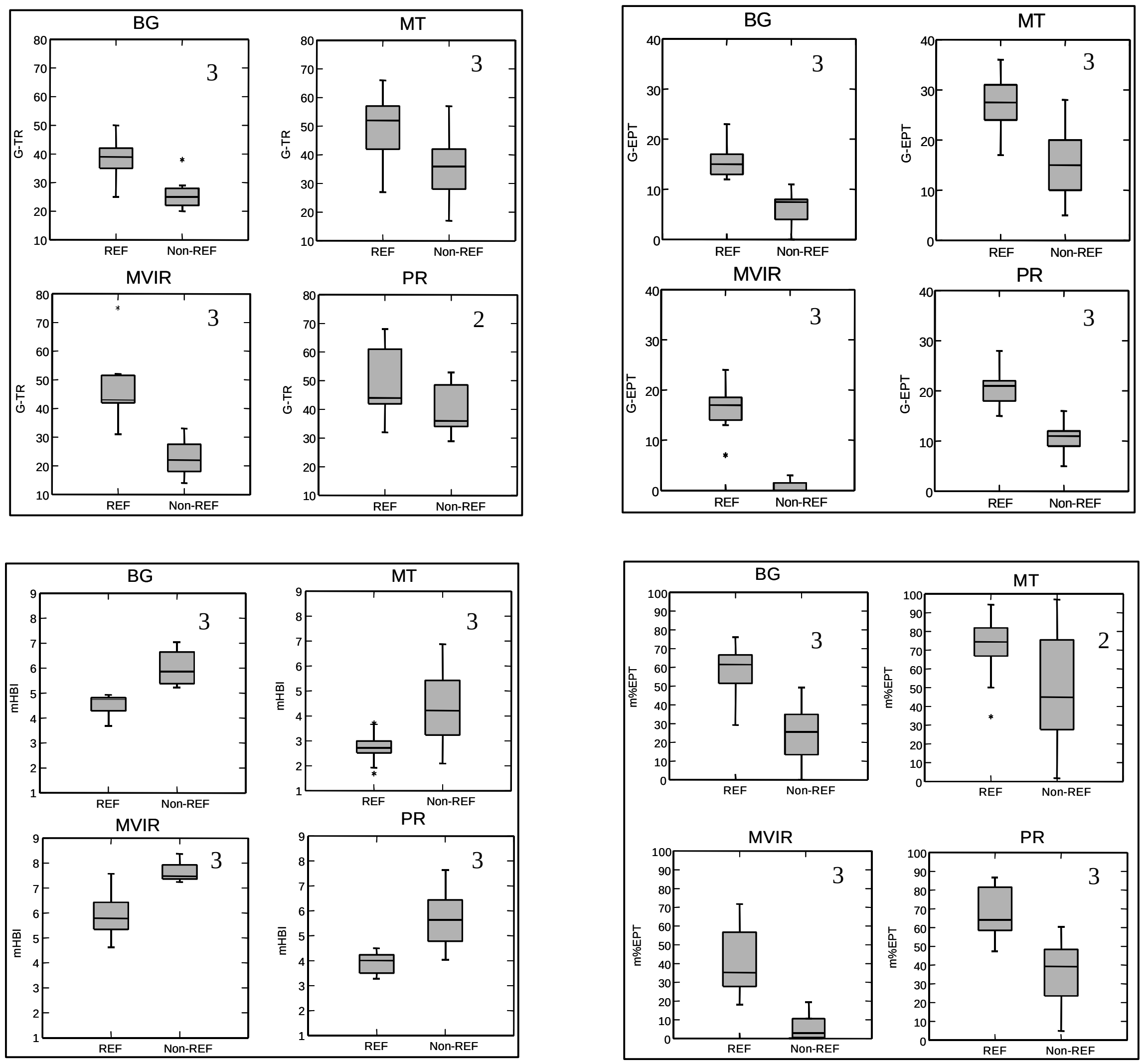

Figure 7. Box plots showing discriminatory power for Genus Taxa Richness (G-TR), Genus EPT (G-EPT), mHBI and m%EPT in headwater reference (REF) and non-reference (Non-REF) streams. Scores in the upper right-hand location of each plot correspond to sensitivity scores (see Figure 2).

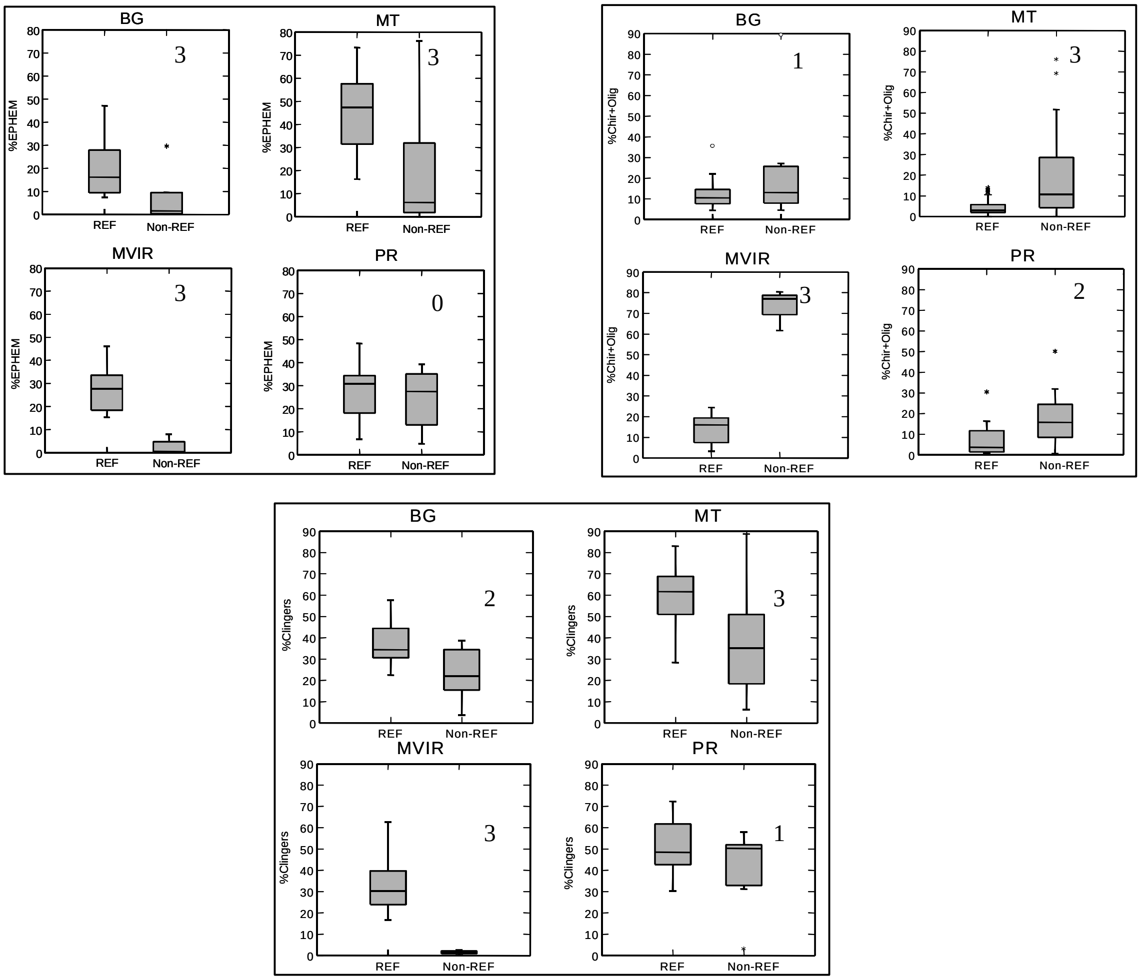

Figure 7 (continued). Box plots showing discriminatory power for %Ephem, %Clingers and %Chir+Olig in headwater reference (REF) and non-reference (Non-REF) streams. Scores in the upper right-hand location of each plot correspond to sensitivity scores (see Figure 2).

The redundancy analysis indicated that none of the seven metrics had correlation coefficients above the 0.75 target in either wadeable or headwater reference streams (Table 13). Pearson correlations ranged from ±0.01 to 0.75, implying that metrics were indeed contributing different information about the community. While TR and EPT had the highest linear correlation in headwater streams (r=0.75) and may provide redundant information, we believe these two metrics should be considered independently. This is due, in part, to the emphasis that both society and resource managers place on total richness as a measure of stream biodiversity (Maxted et al. 2000). We also think that TR offers insight into habitat diversity and niche partitioning. Moreover, in mildly stressed communities, EPT richness may decline while TR increases as facultative and tolerant taxa colonize the stream, and this response signature can help in interpreting bioassessment data.

Table 13. Pearson correlation matrix of statewide reference metric values for wadeable and headwater streams.

Wadeable Reference

	G-TR	*G-EPT*	*mHBI*	*m%EPT*	*%Chir+Olig*	*%Clng*
G-TR	1.00					
G-EPT	0.62	1.00				
mHBI	-0.12	-0.70	1.00			
m%EPT	0.21	0.63	-0.68	1.00		
%Chir+Olig	0.15	-0.12	0.37	-0.26	1.00	
%Clng	0.01	0.40	-0.67	0.36	-0.42	1.00

Headwater Reference

	G-TR	*G-EPT*	*mHBI*	*m%EPT*	*%Ephem*	*%Chir+Olig*	*%Clng*
G-TR	1.00						
G-EPT	0.75	1.00					
mHBI	-0.15	-0.64	1.00				
m%EPT	-0.07	0.30	-0.65	1.00			
%Ephem	0.08	0.39	-0.55	0.66	1.00		
%Chir+Olig	0.21	-0.19	0.57	-0.63	-0.41	1.00	
%Clng	0.26	0.52	-0.54	0.44	0.51	-0.36	1.00

The stressor response analysis revealed that MBI metrics responded predictably to perceived stress (i.e., nutrient enrichment and habitat degradation). All metrics were significantly correlated (p<0.01) with TN, TP and TN*TP (Table 14). Individually, nitrate accounted for the least variance while TP generally accounted for the most. The interactive term (TN*TP) generated the highest correlations for EPT, mHBI and m%EPT. The %Chir+Olig and %Clinger metrics responded well to an ammonia threshold (r = 0.53 and -0.30, respectively), and excessive ammonia was best detected by the mHBI (r = 0.55). %Clingers were the least responsive of all other metrics analyzed with nutrients but responded well to excessive ammonia. For comparison, Figure 8 shows metrics responding to increasing nutrient concentrations (as nutrient codes as defined in Section 4.3).

With regard to habitat, all metrics showed significant (p<0.01) Spearman correlations to most RBP habitat parameter scores (Table 15). The highest correlates included embeddedness, epifaunal substrate, riparian zone width, frequency of riffles and velocity/depth regime. Both EPT and mHBI correlated best with Velocity/Depth Regime score (r = 0.60 and -0.64, respectively). However, most other metrics responded well to this habitat parameter, which suggests that macroinvertebrate communities are possibly enhanced by habitat diversity driven by variations in current velocity and stream depths. For comparison, Figure 9 depicts changes in metric values among habitat stress codes defined in Section 4.3. While this categorical system documents metric responsiveness, Bryce et al. (1999) showed that other variables (e.g., % landcover types, road density, riparian tree size, streamside residential density) should be combined in a more comprehensive stressor risk analysis. In addition, Pond and McMurray (2002) found that conductivity, pH, habitat score, %embeddedness and canopy cover strongly contributed to macroinvertebrate community health in headwater MT streams in Kentucky. Obviously, multiple anthropogenic and natural stressors can operate synergistically on biological assemblages. Hence, future studies on biological response of modified landscapes and chemical attributes in Kentucky are warranted.

Table 14. Pearson correlation matrix of nutrients and macroinvertebrate metrics. Bolded values are **not** significantly different (p>0.01). TKN=Total Kjeldhal Nitogen, TN=Total Nitrogen, TP=Total Phosphorus.

	Ammonia	*Nitrate-N*	*TKN*	*TN*	*TP*	*TN*TP*
TR	-0.39	**-0.20**	-0.27	-0.36	-0.52	-0.50
EPT	-0.48	-0.27	-0.46	-0.52	-0.67	-0.67
mHBI	0.55	0.31	0.51	0.60	0.59	0.64
m%EPT	-0.48	-0.28	-0.56	-0.57	-0.58	-0.64
%Ephem	-0.40	-0.21	-0.49	-0.47	-0.39	-0.41
%Chir+Olig	0.53	**0.11**	0.36	0.32	0.31	0.33
%Clingers	-0.30	**-0.20**	**-0.15**	-0.27	-0.23	-0.27

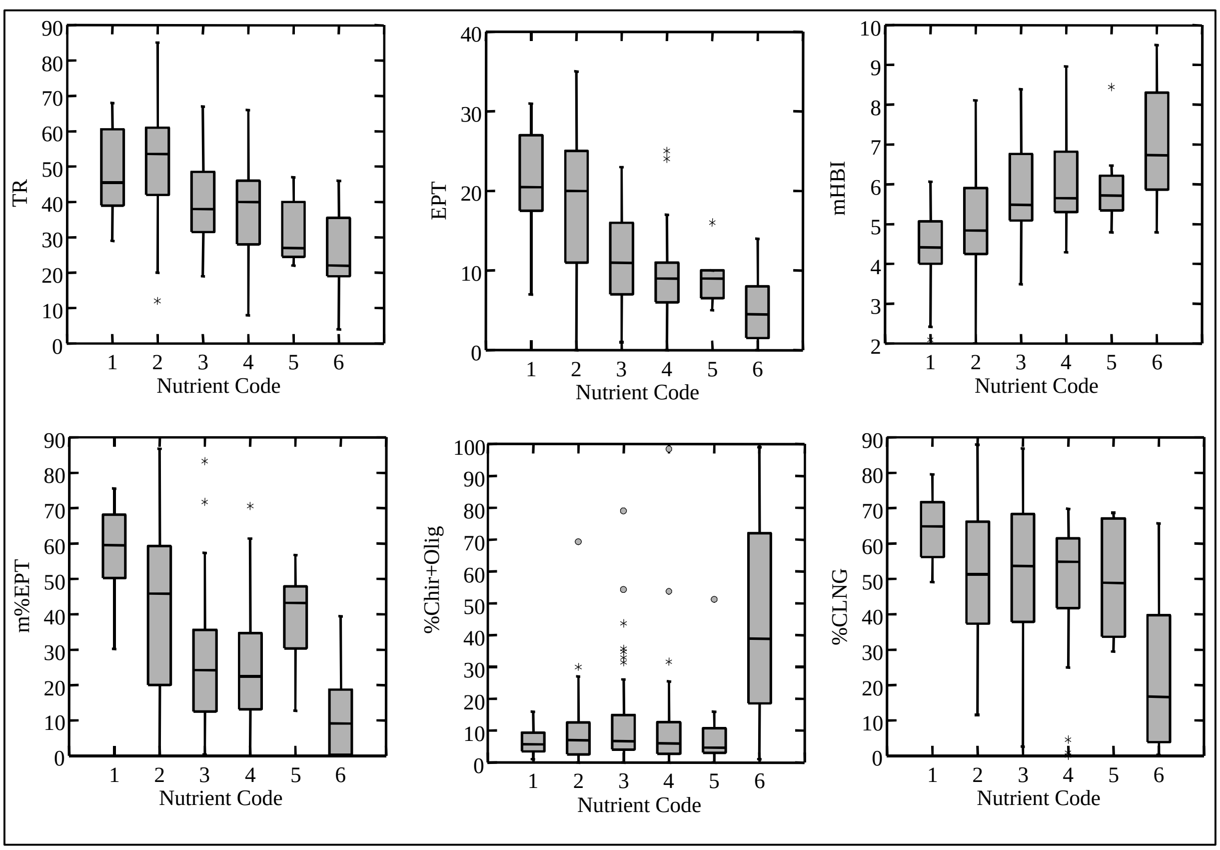

Figure 8. Metric responses to nutrient enrichment categorized as nutrient codes. See Table 6 for code designation.

Table 15. Spearman correlation matrix for all RBP habitat parameter scores and MBI metrics. Bolded values are **not** significantly correlated (*p*< 0.01).

Metric	Embeddedness	Epifaunal Sub	Sediment Dep	Bank Stability	Bank Veg Prot	Riparian Zone	Channel Flow	Chan Alteration	Frequency of Riffles	Velocity/Depth Regime	Pool Variability	Pool Substrate Character	Chan Sinuosity
TR	0.36	0.40	0.27	0.19	0.28	0.23	**0.17**	0.24	0.34	0.48	0.44	0.36	0.37
EPT	0.50	0.56	0.41	0.28	0.40	0.40	0.25	0.32	0.51	0.60	0.40	0.21	0.49
mHBI	-0.44	-0.58	-0.35	-0.31	-0.41	-0.48	-0.34	-0.36	-0.58	-0.64	-0.28	-0.18	-0.36
m%EPT	0.37	0.43	0.33	0.24	0.36	0.38	0.19	0.26	0.46	0.40	**0.13**	0.17	0.17
%Chir+Olig	-0.31	-0.42	-0.25	-0.26	-0.26	-0.22	-0.27	-0.15	-0.40	-0.54	-0.38	**-0.12**	**0.04**
%Clingers	0.27	0.39	0.19	0.17	0.27	0.25	0.20	0.24	0.40	0.54	0.30	**0.05**	0.36

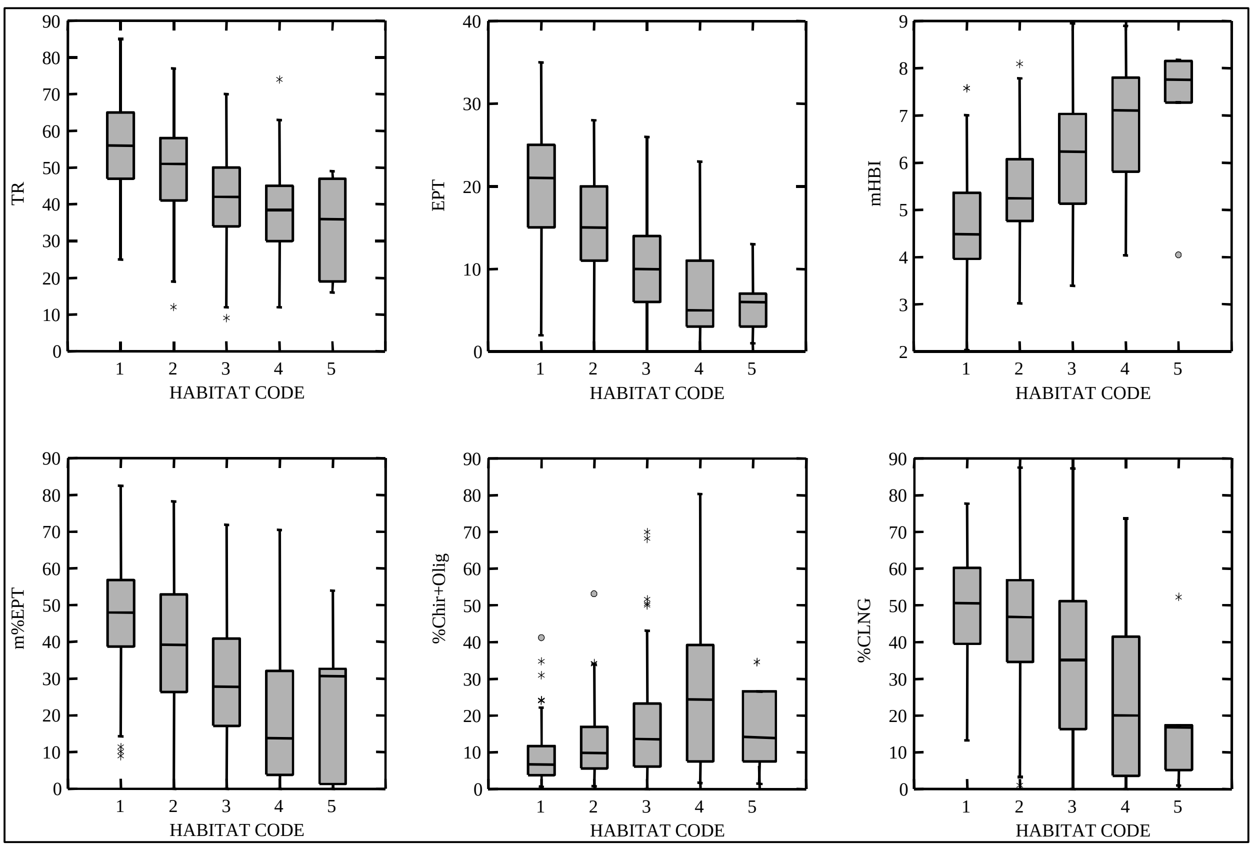

Figure 9. Metric responses to habitat stress categorized as habitat codes. See Table 7 for code designation.

5.4 Metric Scoring and Index Development

Scoring formulae for wadeable and headwater streams, using the 95th percentiles of raw or inverted metrics, are provided by example in Tables 16 and 17, respectively. Formulae used statewide 95th percentile values instead of regional values. Regional criteria for index scores are discussed in Section 5.7.

Table 16. Example MBI calculation for wadeable streams.

Metric	95th or 5th %ile	Formula	Example for Kinniconick Creek	Metric Score
Genus TR	74	$\frac{TR}{95th\%ile}X100$	$\frac{53}{74}X100$	71.62
Genus EPT	30	$\frac{EPT}{95th\%ile}X100$	$\frac{19}{30}X100$	63.33
mHBI	3.11	$\frac{10-mHBI}{10-5th\%ile}X100$	$\frac{10-4.49}{10-3.11}X100$	80.03
m%EPT	74	$\frac{m\%EPT}{95th\%ile}X100$	$\frac{79.69}{74}X100$	100.0
%Chir+Olig	1.0	$\frac{100-\%Chir+Olig}{100-5th\%ile}X100$	$\frac{100-5.04}{100-1.0}X100$	95.92
%Clingers	74	$\frac{\%Clingers}{95th\%ile}X100$	$\frac{60.45}{74}X100$	81.69
MBI			**Average Score =**	**82.09**

Table 17. Example MBI calculation for headwater streams.

Metric	95th or 5th %ile	Formula	Example for UT Flat Creek	Metric Score
Genus TR	63	$\frac{TR}{95th\%ile}X100$	$\frac{39}{63}X100$	61.9
Genus EPT	33	$\frac{EPT}{95th\%ile}X100$	$\frac{15}{33}X100$	45.45
mHBI	2.18	$\frac{10-mHBI}{10-5th\%ile}X100$	$\frac{10-4.59}{10-2.18}X100$	69.18
m%EPT	86.9	$\frac{m\%EPT}{95th\%ile}X100$	$\frac{62.2}{86.9}X100$	71.57
%Ephem	66.5	$\frac{\%Ephem}{95th\%ile}X100$	$\frac{8.93}{66.5}X100$	13.43
%Chir+Olig	0.68	$\frac{100-\%Chir+Olig}{100-5th\%ile}X100$	$\frac{100-4.47}{100-0.68}X100$	92.31
%Clingers	75.5	$\frac{\%Clingers}{95th\%ile}X100$	$\frac{25.1}{75.5}X100$	33.01
MBI			**Average Score =**	**55.34**

5.5 MBI Performance and Sensitivity

The ability of the wadeable and headwater MBI to regionally discriminate between reference and non-reference streams is depicted in Figures 10 and 11, respectively. These box plots show that the index has excellent discriminatory power in all bioregions. This, in conjunction with the narrow interquartile ranges of reference sites (<10 points), indicates that reference sites were well chosen (i.e., greater biological performance) and that there is relatively low variability in the reference condition as expressed by the MBI. Reference MBI scores for each site are listed in Appendix B through E.

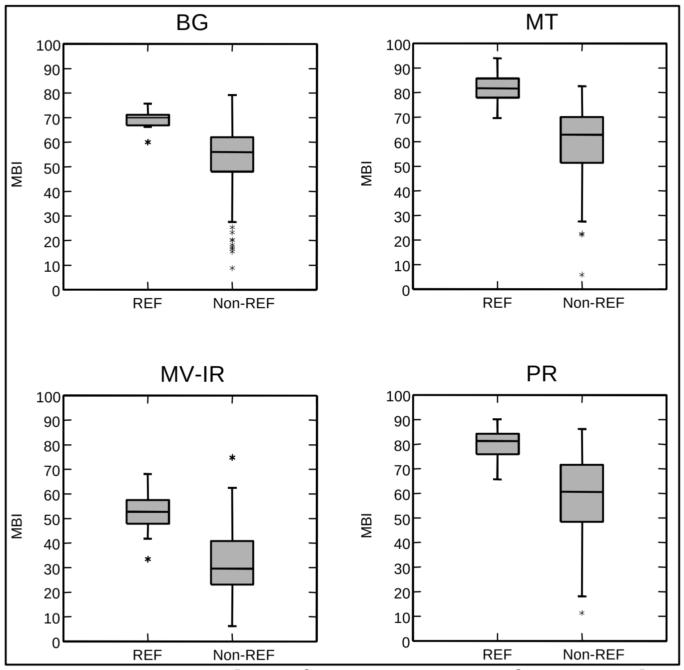

Figure 10. Box plots of MBI scores at reference and non-reference wadeable sites by bioregion.

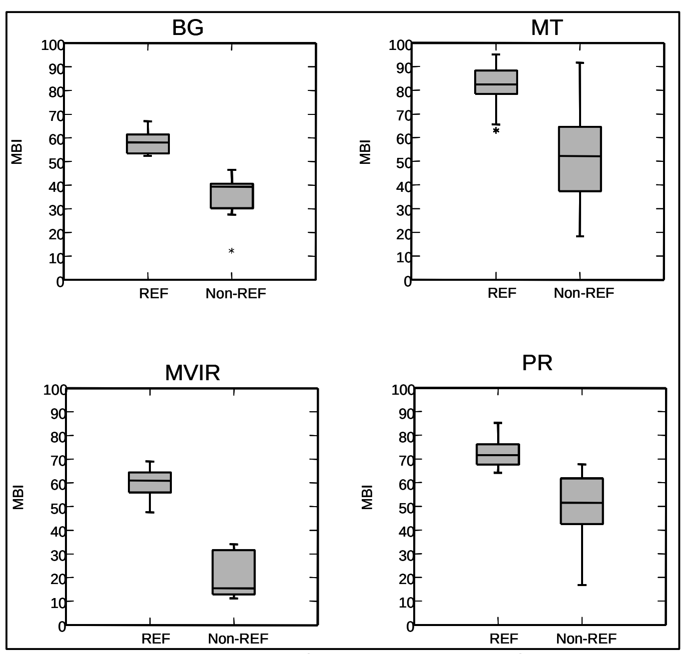

Figure 11. Box plots of MBI scores at reference and non-reference headwater sites by bioregion.

The aggregate MBI also showed good response to increasing nutrient concentrations and habitat degradation. MBI scores were significantly correlated (r = -0.64, p < 0.0001) with the interactive term (TN*TP) (Figure 12) and also showed good response among the six nutrient codes (Figure 13). With regard to habitat, the MBI was also highly correlated with RBP Habitat scores (r = 0.65, p <0.0001) (Figure 14) and responded predictably among the habitat stress codes (Figure 15). These results are promising in that the index could track nutrient and habitat stressors, stressors that currently account for more than 50% of the stream segments listed as impaired in Kentucky (KDOW 2000b).

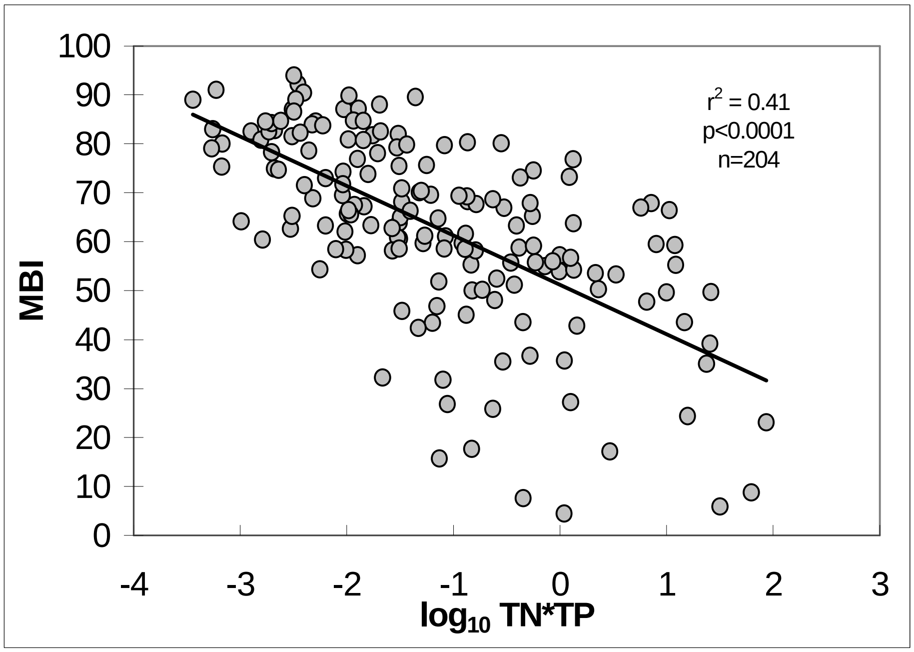

Figure 12. Scatter plot of statewide MBI scores vs. log10 Total Nitrogen * Total Phosphorus.

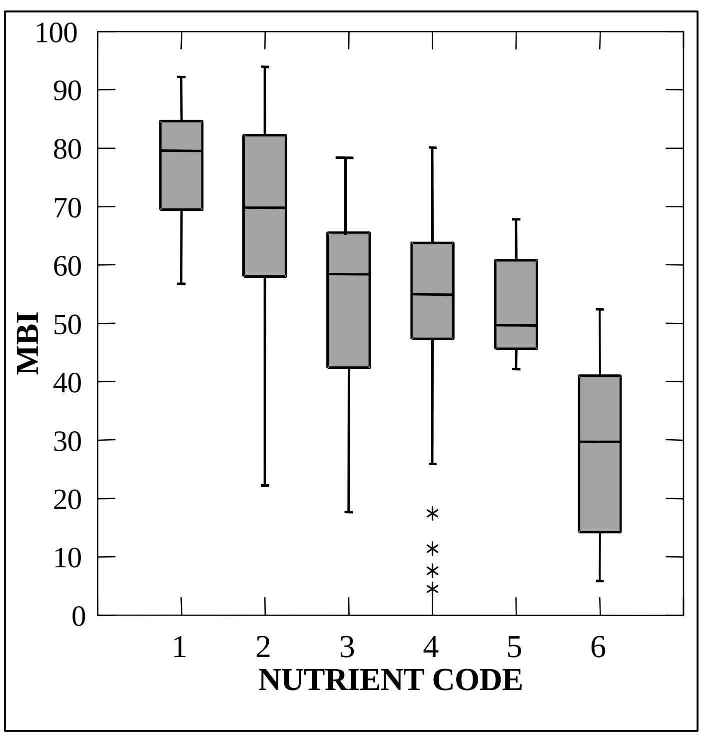

Figure 13. Box plot of statewide MBI scores vs. nutrient codes. See Table 6 for code designations.

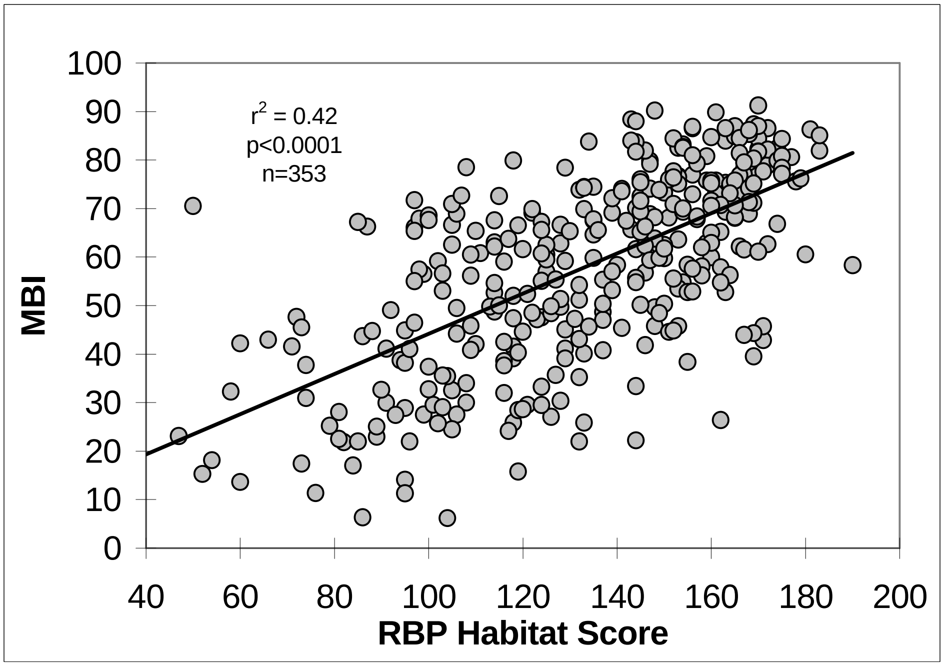

Figure 14. Scatter plot of statewide MBI scores vs. total RBP habitat scores.

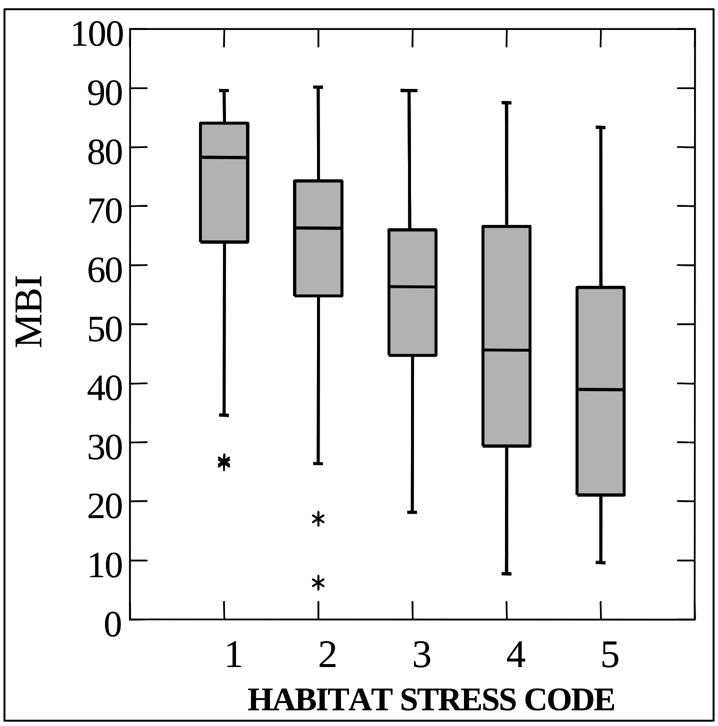

Figure 15. Box plot of statewide MBI scores vs. habitat codes. See Table 7 for code designations.

The response of the MBI to nutrient and habitat stressors by bioregion is depicted in Figures 16 and 17, respectively. For the nutrient gradient, all bioregions except the BG showed either a gradual or sharp decline in MBI scores. The apparent failure of the MBI to detect nutrient enrichment among codes 2 through 5 in the BG is interesting and might be attributable to regional faunal characteristics

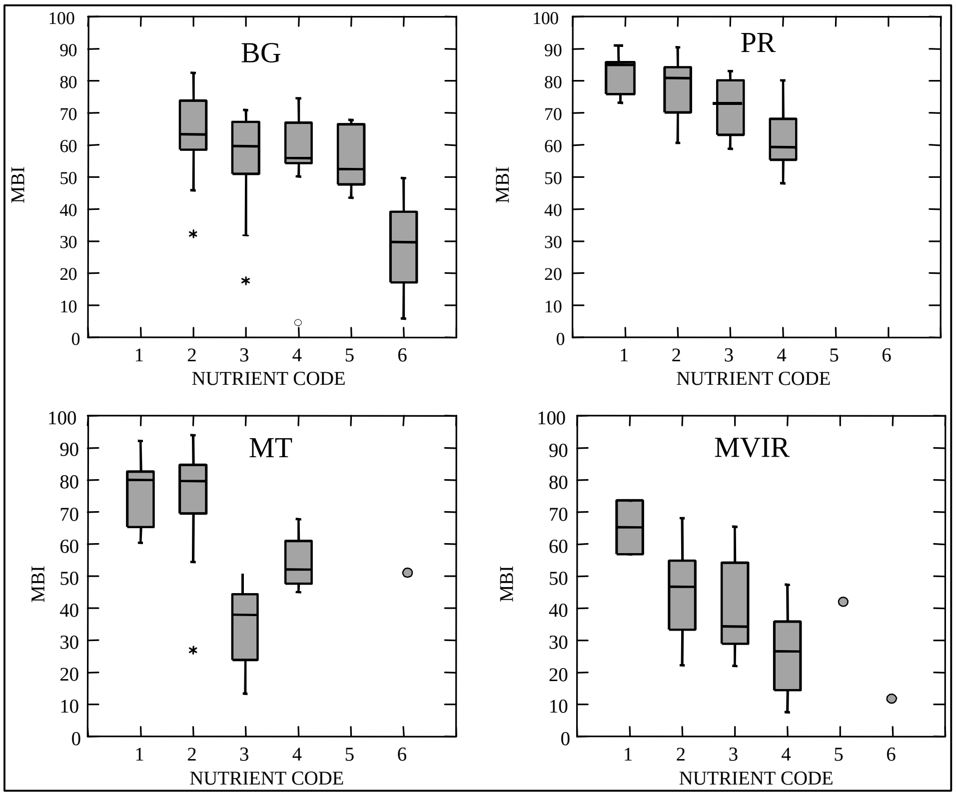

Figure 16. Boxplots of MBI scores vs. nutrient codes, by bioregion.

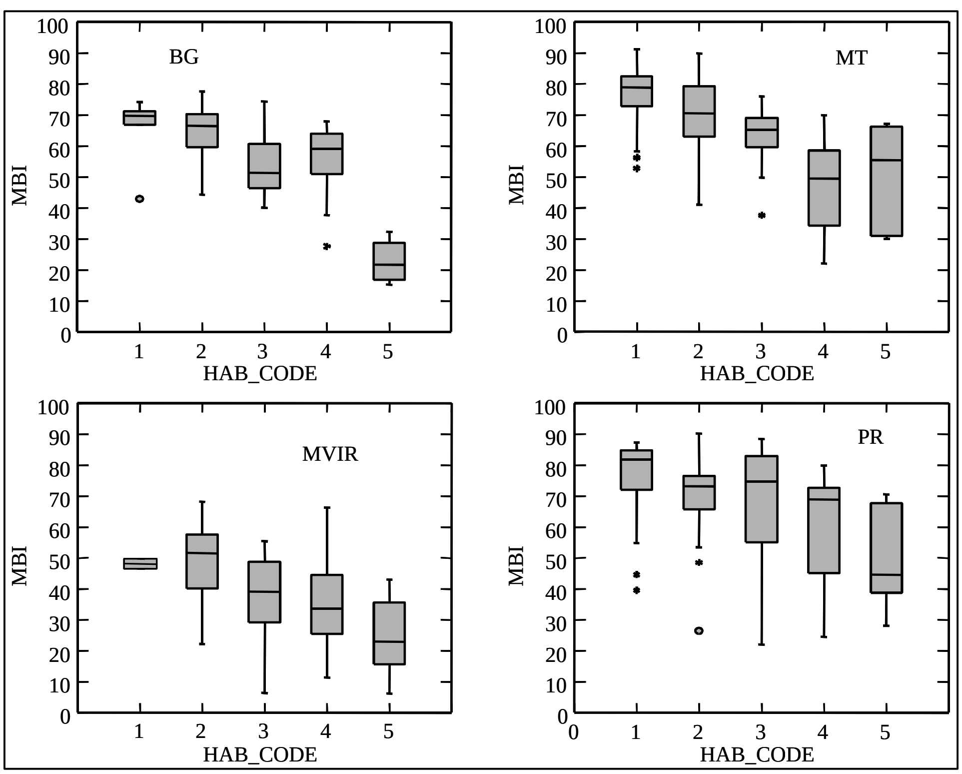

Figure 17. Boxplots of MBI scores vs. habitat stress codes, by bioregion.

unique to the Blue Grass. One hypothesis is that benthic algal and macroinvertebrate communities experience naturally occurring phosphorus concentrations (from phosphatic Ordovician lithology) that are above saturation level for these communities. BG fauna are thus perhaps adapted to deleterious effects caused by elevated nutrient concentrations. Another possibility is that the region experiences hydrological stress. For example, even low-nutrient streams with good instream habitat are hydrologically unstable (i.e., drought-prone, intermittent/interrupted) in this region. This can lead to excessive temperatures and reduced dissolved oxygen (D.O.) concentrations for extended periods throughout the summer months. Comparatively, nutrient enrichment can also indirectly lead to diel sags in D.O. due to increased biological oxygen demand or respiration of increased biomass. It is probable that the BG invertebrate fauna are thus naturally facultative or tolerant to nutrient enrichment (as expressed in higher tolerance values, fewer sensitive species, more colonizers). The BG MBI scores did show a strong response to high instream ammonia concentrations (>1.0 mg/l) indicating the toxic nature of this stressor.

Bioregional MBI scores showed a better relationship to instream habitat degradation where most of the best streams (i.e., as expressed by the MBI) had habitat stress codes of 1 or 2, compared to the worst MBI scores in those streams rating a 4 or 5.

Elevated conductivity (a surrogate for total dissolved solids arising mainly from coal mining activities) was also found to affect MBI scores in the MT headwater sites (Figure 18). It was apparent that this stressor compromised biological integrity in these small streams. Conductivity will be evaluated in the future in other bioregions in both wadeable and headwater systems.

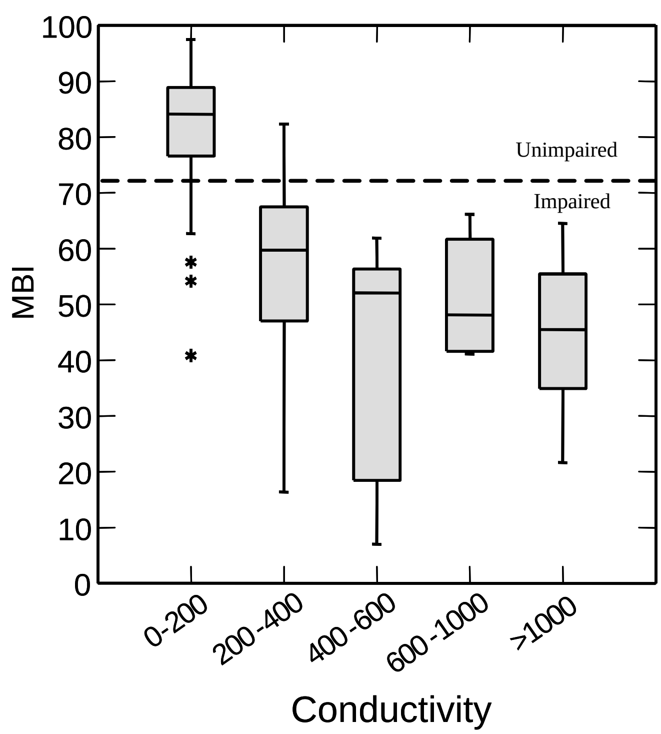

Figure 18. Box plot of MBI scores from MT headwater streams graphed against increasing conductivity (µS/cm). The dotted line indicates the impairment threshold (see Table 18).

5.6 Index Precision and Relationship to Drainage Area

A check on the repeatabilty of MBI scores was done at 15 reference sites scattered throughout all bioregions. Figure 19 demonstrates the correlation between initial and revisit MBI scores. This analysis may suggest that collection and assessment methods are consistent and that the MBI is repeatable. The average MBI difference among these repeated observations was 2.9 points, ranging from 0.1 to 7.1 points.

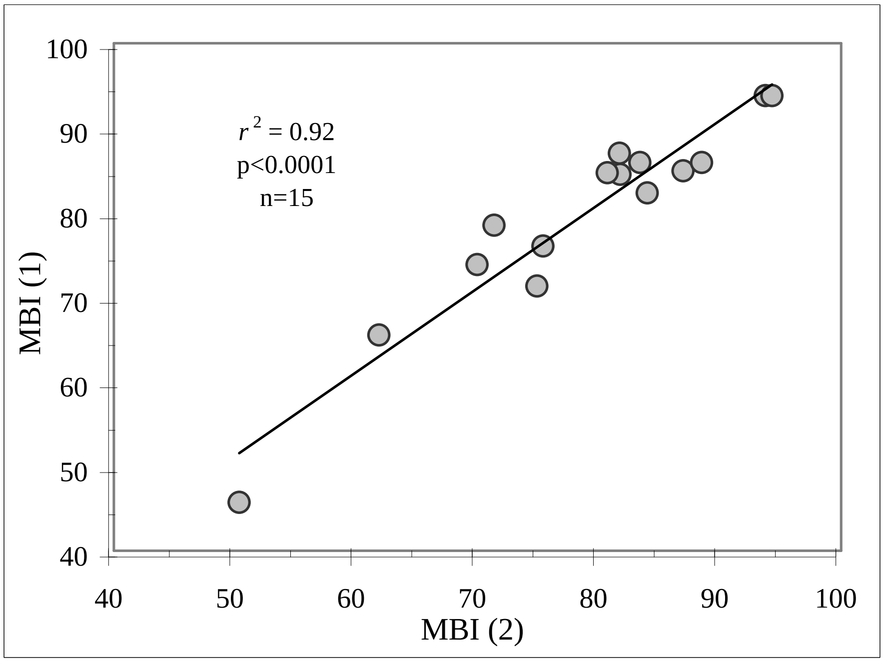

Figure 19. Linear regression of initial (MBI 1) and revisit (MBI 2) index scores.

In terms of the influence of drainage area on MBI scores, simple linear regression detected no significant effect, except for headwater PR streams where MBI scores increased with drainage area (r^2=0.39, p<0.05). Nonsignificant r^2-values for the other three headwater bioregions and the four wadeable bioregions ranged from 0.001 to 0.12 with p-values >0.14. Additional data are needed to better undestand drainage area influences in PR headwater streams.

5.7 MBI Application and Narrative Criteria

The application of the MBI involves comparing scores from new sites to condition classes or narrative ratings derived from the statistical distribution of scores found at regional reference sites. Narrative ratings were assigned to individual sites based on a combination of percentile distributions and trisection of the reference MBI scores (100-point scale). While the use of the 25th %ile of reference data is often used as a biocriterion (see Barbour et al. 1999), the WQB recognizes that many reference streams in the MVIR bioregion are more physically or chemically stressed than in reference sites in the other three regions, and this warrants the use of region-specific percentile cutoffs. This rationale also implies that many reference sites in the MT and PR regions and some BG streams are considered "*minimally*-impacted" (i.e., mostly forested watersheds, natural channel pattern), whereas those in the MVIR are more appropriately deemed "*least*-impacted" (i.e., best available considering current and legacy land uses). Furthermore, we felt that the narrow interquartile range of MBI scores warranted alternative %ile cutoffs. Table 18 lists regional narrative thresholds for Excellent, Good, Fair, Poor and Very Poor ratings. Exceptional Water criteria are based on the 50th %ile for MT, BG and PR reference streams and the 75th %ile for MVIR streams. Streams rating "Good" in MT, BG and PR regions score at or above the 5th %ile whereas MVIR sites need to score at or above the 25th %ile. Trisection of scores below this value (i.e., at the 5th or 25th %ile) was used to designate Fair, Poor and Very Poor ratings.

Table 18. MBI criteria for assigning narrative ratings for wadeable (a) and headwater streams (b) by bioregion. Based on either 75th/25th %ile or 50th/5th %ile cutoffs for “Excellent” and “Good” and further trisection of values below a rating of "Good".

Wadeable	50th and 5th %ile	50th and 5th %ile	50th and 5th %ile	75th and 25th %ile
Rating	BG	MT	PR	MVIR
Excellent	$\geq$ 70	$\geq$ 82	$\geq$ 81	$\geq$ 58
Good	61–69	75–81	72–80	48–57
Fair	41–60	50–74	49–71	24–47
Poor	21–40	25–49	25–48	13–23
Very Poor	0–20	0–24	0–24	0–12

Headwater

Rating	BG	MT	PR	MVIR
Excellent	$\geq$ 58	$\geq$ 83	$\geq$ 72	$\geq$ 63
Good	51–57	72–82	65–71	56–62
Fair	39–50	48–71	43–64	35–55
Poor	19–38	24–47	22–42	19–34
Very Poor	0–18	0–23	0–21	0–18

5.8 Conclusions and Future Directions

Kentucky's revised MBI and its associated metrics appear to be both robust and repeatable in headwater and wadeable streams. The aggregate index will be used to rate water quality conditions of streams and also to identify those highest quality waters or “Exceptional Waters” deserving stricter protection under Kentucky's antidegradation regulations. In cases when MBI scores fall close to narrative rating thresholds, caution should be used in the rating, and a re-sample of the site may be warranted. While we are confident that the MBI can be used as a “stand-alone” assessment tool, any additional data (e.g., fish, algal, habitat, chemical) should be used in conjunction with the MBI for a more thorough weight-of-evidence approach. To be effective, both the sampling protocol and sample index periods should be closely followed, and sites should be classified using the bioregion map in Appendix A. Future studies may include: (1) reference site expansion into all Level IV ecoregions; (2) sampling at different times of the year to determine seasonal variability of reference communities; and (3) testing the effects of other chemical, nutrient and physical stressors at regional scales to define and understand effect levels and biological response signatures (Yoder and Rankin 1995).

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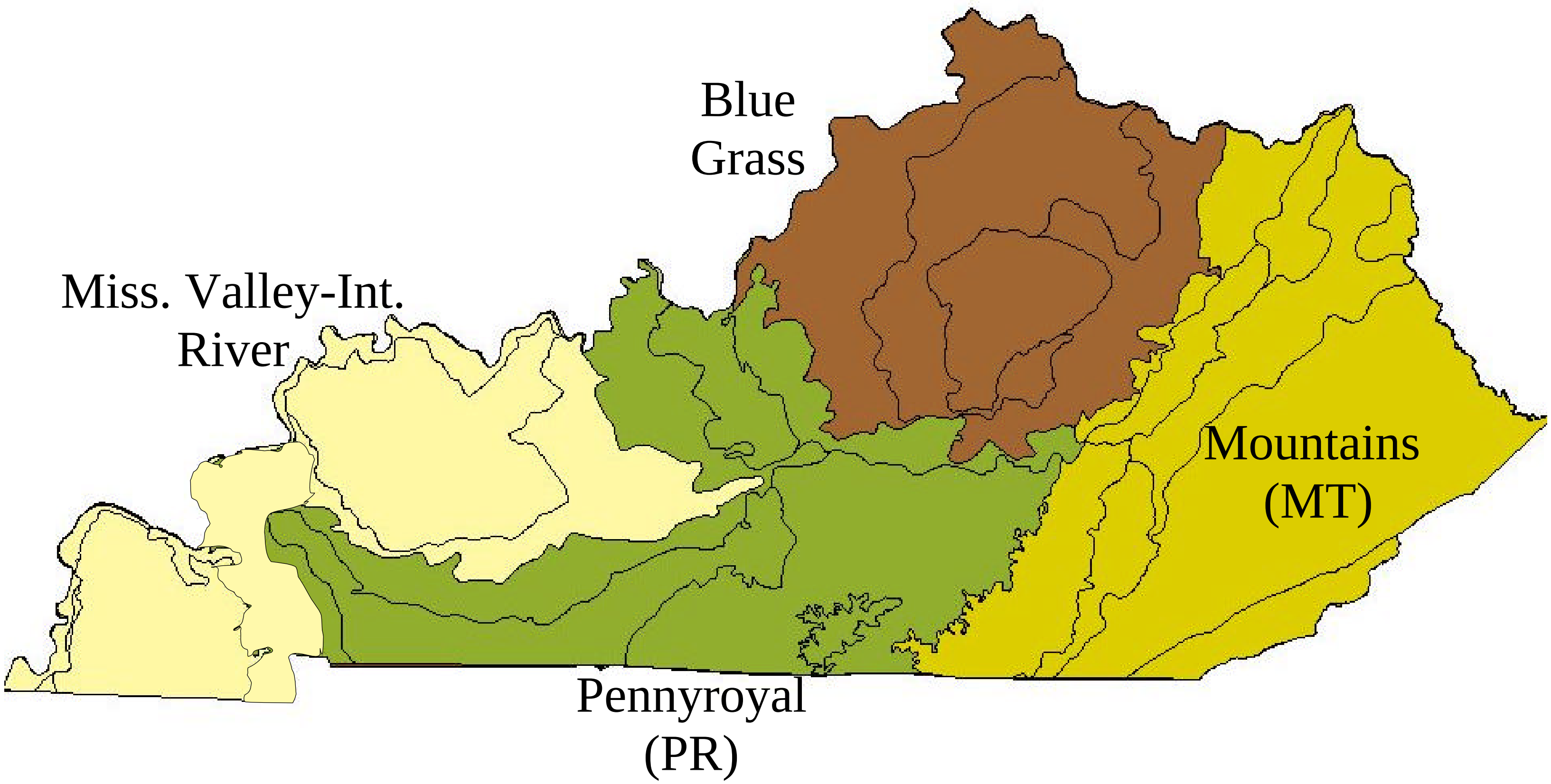
APPENDICES



Appendix A. Map of the four-bioregion classification used for macroinvertebrate assessments. Solid lines refer to Level IV subecoregions (after Woods et al. 2002). Investigators should use best professional judgment when sample sites fall near region lines.

Appendix B. Metric and MBI values for Blue Grass (BG) wadeable and headwater sites.

Wadeable

StationID	StreamName	CollDate	Sub-Ecoregion	Bioregion	Basin	Order	Drainage Area	G-TR	G-EPT	mHBI	m%EPT	%C+O	%ClngP	TotInd	MBI
CFD04005504	SEVERN CREEK	6/20/02	71k	BG	KENTUCKY	4	30.50	60	21	5.26	54.0	7.6	54.7	1200	75.71
DOW04004009	MILL CREEK	6/13/02	71k	BG	KENTUCKY	4	11.60	49	20	5.36	43.0	10.7	51.3	1221	68.10
DOW04005006	SIXMILE CREEK	6/19/02	71k	BG	KENTUCKY	4	42.10	67	21	5.49	31.5	18.0	55.5	1208	68.24
DOW04010006	MUSSELMAN CREEK	6/12/02	71k	BG	KENTUCKY	3	8.00	48	19	4.92	57.4	5.6	40.2	1137	70.90
DOW04014012	CLEAR CREEK	7/10/98	71l	BG	KENTUCKY	4	61.60	57	18	4.78	29.4	1.9	36.9	924	66.84
DOW04014022	GRIER CREEK	6/18/02	71l	BG	KENTUCKY	4	13.30	49	24	4.37	33.5	8.5	50.7	2365	71.16
DOW04036005	DROWNING CREEK	8/ 4/98	71d	BG	KENTUCKY	3	17.00	71	20	5.97	31.1	6.1	34.3	890	66.61
DOW05009001	S. F. GRASSY CREEK	6/1/99	71k	BG	LICKING	4	30.70	55	21	4.71	35.0	6.4	62.7	534	73.82
DOW05009002	S. F. GRASSY CREEK	7/12/99	71k	BG	LICKING	4	45.20	54	17	5.56	29.8	2.8	65.6	315	69.97
DOW05028007	WEST CREEK	6/1/99	71k	BG	LICKING	3	9.60	44	17	5.13	39.5	4.5	66.7	375	70.64
DOW12004001	CEDAR CREEK	7/15/99	71d	BG	SALT	3	12.20	48	14	5.47	30.9	4.1	34.6	486	59.98
DOW12023001	CHAPLIN RIVER	7/ 8/99	71k	BG	SALT	4	116.70	50	16	4.47	39.8	1.7	68.4	766	74.45
DOW12023003	SULPHUR CREEK2	6/10/99	71k	BG	SALT	4	21.90	37	12	4.52	29.7	4.5	70.1	465	66.28

Headwater

StationID	StreamName	CollDate	Sub-Ecoregion	Bioregion	Basin	Order	Drainage Area	G-TR	G-EPT	mHBI	m%EPT	%Ephem	%C+O	%ClngP	TotInd	MBI
DOW04022009	HINES CREEK	2/25/2002	71l	BG	KENTUCKY	2	2.07	39	16	4.20	51.4	7.5	19.4	46.4	494	56.8
DOW04012003	GRINDSTONE CREEK	3/11/2002	71k	BG	KENTUCKY	2	1.80	48	23	4.05	60.4	14.8	11.3	58.3	2060	68.6
DOW04005008	CEDAR CREEK UT	3/12/2003	71k	BG	KENTUCKY	1	0.78	32	13	4.63	61.5	0.1	5.7	52.1	1266	56.3
DOW08066001	BIG SUGAR CREEK UT	3/14/2003	71d	BG	OHIO	2	2.18	34	14	4.59	51.6	1.0	28.0	49.9	1977	52.1
DOW04007003	INDIAN CREEK2	3/25/2003	71d	BG	KENTUCKY	3	5.60	39	15	4.54	38.4	5.2	25.1	54.0	705	53.8
DOW04007003	INDIAN CREEK2	4/8/1999	71d	BG	KENTUCKY	3	5.60	27	13	4.69	66.0	33.3	8.9	29.6	291	58.2
DOW04012004	KENTUCKY RIVER UT	3/29/2002	71k	BG	KENTUCKY	1	0.65	39	16	4.20	70.6	9.5	7.5	55.5	1604	63.8
DOW08073003	ASHBYS FORK	3/6/2002	71d	BG	OHIO	2	2.20	41	16	4.84	35.4	15.9	22.2	37.6	923	53.2
DOW08057003	CORN CREEK UT	4/11/2002	71d	BG	OHIO	1	0.95	34	14	3.59	61.5	29.3	12.5	22.5	1124	58.7
DOW04006002	FLAT CREEK UT	4/14/2002	71k	BG	KENTUCKY	1	0.65	39	15	4.59	62.2	8.9	4.5	25.1	582	55.9
DOW08074003	SECOND CREEK	4/15/2003	71d	BG	OHIO	2	2.20	36	15	4.44	50.5	3.7	34.0	48.2	1549	52.5
DOW04013032	GLENNS CREEK UT	4/2/2003	71l	BG	KENTUCKY	2	0.80	51	20	4.45	70.0	30.2	4.9	25.1	2892	66.8
DOW04005004	BACKBONE CREEK	4/25/2001	71k	BG	KENTUCKY	3	6.10	50	20	4.93	29.2	16.2	35.7	48.9	1265	56.1
DOW04005005	LITTLE SIXMILE CREEK	4/25/2001	71k	BG	KENTUCKY	2	4.60	43	20	4.46	45.8	19.6	14.5	53.7	1006	62.7
DOW04006001	SAND RIPPLE CREEK	4/4/2001	71k	BG	KENTUCKY	2	4.50	28	12	4.81	65.5	7.9	10.5	31.3	466	52.3
DOW04006001	SAND RIPPLE CREEK	4/8/1999	71k	BG	KENTUCKY	2	4.50	25	12	3.76	77.7	29.6	8.6	22.3	233	58.8
DOW04012002	DUVALL BRANCH	4/4/2001	71k	BG	KENTUCKY	1	0.84	36	13	4.79	59.7	20.9	5.2	30.6	853	57.0
DOW08073004	DOUBLE LICK CREEK	5/28/2003	71d	BG	OHIO	2	2.31	34	15	4.84	50.0	26.9	6.7	46.6	416	58.6
DOW08074002	GARRISON CREEK	5/4/2000	71d	BG	OHIO	3	4.50	38	14	4.72	70.0	47.1	7.7	35.7	911	66.0

Appendix C. Metric and MBI values for Mountain (MT) wadeable and headwater sites.

Wadeable

StationID	StreamName	CollDate	Bioregion	Sub-Ecoregion	Basin	Order	Drainage Area	G-TR	G-EPT	mHBI	m%EPT	%C+O	%ClngP	TotInd	MBI
DOW02006022	LITTLE SOUTH FORK	7/14/00	MT	68c	UPPER CUMBERLAND	4	50.1	72	27	4.07	46.1	6.3	60.0	568	85.4
DOW02006023	CANE CREEK	6/30/00	MT	68c	UPPER CUMBERLAND	3	7.9	69	23	3.46	40.6	6.5	61.7	431	83.0
DOW02006024	BARK CAMP CREEK	6/23/00	MT	68c	UPPER CUMBERLAND	4	9.9	65	31	3.66	65.3	6.1	43.7	412	87.2
DOW02006026	EAGLE CREEK	7/ 5/00	MT	68c	UPPER CUMBERLAND	3	4.6	56	21	3.93	47.9	9.3	64.6	560	79.7
DOW02006028	DOG SLAUGHTER CREEK	6/22/00	MT	68c	UPPER CUMBERLAND	4	6.9	62	30	3.48	42.1	7.5	50.9	468	83.0
DOW02006032	BEAVER CREEK	7/17/00	MT	68c	UPPER CUMBERLAND	4	25	58	21	2.72	65.3	5.5	67.2	366	87.4
DOW02006033	S. F. DOG SLAUGHTER CREEK	6/22/00	MT	68c	UPPER CUMBERLAND	3	4.2	58	30	3.35	55.4	8.3	55.2	505	86.3
DOW02008007	ROCK CREEK	7/31/97	MT	68c	UPPER CUMBERLAND	3	19	55	28	3.11	74.2	6.2	49.2	260	88.2
DOW02013001	INDIAN CREEK1	7/ 6/00	MT	68c	UPPER CUMBERLAND	4	26.8	63	26	3.50	46.3	5.2	77.8	445	87.5
DOW02013002	COGUR FORK	7/ 6/00	MT	68c	UPPER CUMBERLAND	3	9.9	47	17	3.92	41.5	3.5	74.1	455	77.1
DOW02014003	MARSH CREEK	7/ 6/00	MT	68c	UPPER CUMBERLAND	5	39	55	20	4.24	68.9	4.0	61.7	373	83.2
DOW02018004	BUNCHES CREEK	7/17/00	MT	68c	UPPER CUMBERLAND	4	8.2	63	28	3.67	49.1	6.9	50.9	350	83.4
DOW02019002	SINKING CREEK	7/18/00	MT	68c	UPPER CUMBERLAND	5	36.1	58	21	4.51	58.1	12.0	52.3	384	77.9
DOW02023002	HORSE LICK CREEK	7/13/00	MT	68c	UPPER CUMBERLAND	4	56.2	54	22	3.67	82.6	0.7	32.1	1831	80.3
DOW02024001	M. F. ROCKCASTLE RIVER	8/ 8/00	MT	68c	UPPER CUMBERLAND	5	85	67	24	4.17	63.3	11.4	47.7	1312	82.6
DOW02024002	LAUREL FORK	7/10/00	MT	68c	UPPER CUMBERLAND	5	31.6	65	21	3.70	59.9	5.5	45.3	419	81.3
DOW02041002	BROWNIES CREEK2	7/20/00	MT	69e	UPPER CUMBERLAND	3	4.7	51	25	3.02	64.2	1.6	80.2	257	89.9
DOW02044001	FUGITT CREEK	7/19/00	MT	69e	UPPER CUMBERLAND	3	5.5	42	26	2.03	58.6	1.8	73.8	336	87.1
DOW02044001	FUGITT CREEK	9/16/99	MT	69e	UPPER CUMBERLAND	3	5.5	51	26	2.50	49.6	1.5	50.1	804	81.8
DOW02046003	POOR FORK CUMBERLAND RIVER	7/19/00	MT	69e	UPPER CUMBERLAND	3	6.1	47	28	2.58	71.5	1.0	68.5	298	91.2
DOW04036010	CAVANAUGH CREEK	7/ 2/98	MT	70g	KENTUCKY	3	12.5	54	25	3.82	65.1	6.2	60.3	390	85.2
DBF04036701	CAVANAUGH CREEK	7/3/2000	MT	70g	KENTUCKY	3	9.7	54	24	3.85	55.1	12.6	51.8	564	79.3
DOW04042009	RED RIVER	8/21/98	MT	70g	KENTUCKY	4	142.2	56	23	4.58	72.0	10.5	49.3	525	81.1
DOW04042011	GLADIE CREEK	8/16/00	MT	70g	KENTUCKY	4	22.7	56	25	3.79	67.1	6.2	56.7	210	85.4
DOW04044001	RIGHT FORK BUFFALO CREEK	8/16/00	MT	69d	KENTUCKY	3	15.1	54	19	4.23	38.7	3.8	69.4	445	77.3
DOW04050003	COLES FORK	8/ 4/99	MT	69d	KENTUCKY	3	6.4	40	16	3.02	39.6	4.8	74.3	187	76.3
DOW04053005	HELL FOR CERTAIN CREEK	8/27/98	MT	69d	KENTUCKY	4	10.5	55	21	4.06	69.0	8.1	38.3	248	78.3
DOW04054001	MIDDLE FORK KENTUCKY RIVER	8/26/98	MT	69d	KENTUCKY	5	198	75	24	4.34	55.6	9.3	43.0	495	81.3
DOW05036001	NORTH FORK LICKING RIVER	7/ 1/99	MT	70f	LICKING	5	36.1	51	19	4.34	67.2	3.4	50.8	1009	78.8
DOW05036003	DEVILS FORK	7/ 1/99	MT	70f	LICKING	4	17.9	69	20	3.84	65.8	6.7	67.9	386	87.6
DOW05038001	BLACKWATER CREEK	6/17/99	MT	70f	LICKING	5	38.2	52	18	4.75	53.2	15.9	64.8	863	75.3
DOW06010002	BIG SINKING CREEK	6/27/02	MT	70h	LITTLE SANDY	4	17.5	56	28	4.32	48.4	17.6	47.4	500	77.5
DOW06013017	LAUREL CREEK	7/ 5/01	MT	70h	LITTLE SANDY	4	14.6	52	27	3.99	55.1	8.3	75.5	325	85.9
DOW06013017	LAUREL CREEK	7/ 2/02	MT	70h	LITTLE SANDY	4	14.6	61	27	4.01	47.7	8.0	54.7	686	81.9
DOW08095004	KINNICONICK CREEK	7/23/02	MT	70d	OHIO	5	87.9	53	19	4.49	79.7	5.0	60.5	1507	82.1
DOW06013003	BIG CANEY CREEK	7/5/2001	MT	70h	LITTLE SANDY	3	11.2	66	30	3.66	75.0	5.3	68.2	768	94.8
DOW06013003	BIG CANEY CREEK	6/25/2002	MT	70h	LITTLE SANDY	3	11.2	65	31	3.55	54.1	10.2	61.2	629	88.1
DOW06013003	BIG CANEY CREEK	6/25/2002	MT	70h	LITTLE SANDY	3	11.2	69	33	3.83	74.0	9.4	66.6	1256	94.1

Appendix C (Continued). Metric and MBI values for Mountain (MT) wadeable and headwater sites.

Headwater

StationID	StreamName	CollDate	Bioregion	Sub-Ecoregion	Basin	Order	Drainage Area	G-TR	G-EPT	mHBI	m%EPT	%Ephem	%C+O	%ClngP	TotInd	MBI
DBF02024705	MILL CREEK	4/18/2001	MT	68a	UPPER CUMBERLAND	2	2.6	46	29	2.70	68.0	25.4	6.8	62.5	1090	78.2
DBF04042703	CHESTER CREEK	4/10/2002	MT	70f	KENTUCKY	2	2.65	58	30	2.42	68.7	32.5	10.2	68.4	332	84.1
DOW01007005	HOBBS FORK	4/11/2001	MT	69d	BIG SANDY	2	1.15	56	31	2.77	78.9	56.4	2.0	70.5	342	91.9
DOW01007006	HOBBS FORK2 UT	4/11/2001	MT	69d	BIG SANDY	1	0.18	48	29	2.18	87.1	55.0	0.9	66.4	464	90.6
DOW01032001	TOMS BRANCH	4/12/2001	MT	69d	BIG SANDY	1	0.95	58	32	2.58	82.5	59.3	3.5	71.8	578	94.3
DOW01032002	LOWER PIGEON BRANCH	4/12/2001	MT	69d	BIG SANDY	1	0.89	53	29	2.55	66.9	42.2	5.6	66.7	673	84.4
DOW01032002	LOWER PIGEON BRANCH	5/15/2002	MT	69d	BIG SANDY	1	0.89	45	30	1.68	91.7	54.1	2.0	55.4	410	88.0
DOW01032002	LOWER PIGEON BRANCH	5/16/2002	MT	69d	BIG SANDY	1	0.89	49	27	2.22	85.9	46.7	2.9	53.6	377	85.3
DOW02006030	JACKIE BRANCH	4/20/2000	MT	68c	UPPER CUMBERLAND	2	1.14	53	25	2.94	62.5	43.4	4.9	70.9	371	82.4
DOW02006031	CANE CREEK	4/24/2000	MT	68c	UPPER CUMBERLAND	1	0.65	52	26	2.66	78.0	32.3	3.6	50.1	449	79.6
DOW02008017	ROCK CREEK1 UT	4/12/2000	MT	68c	UPPER CUMBERLAND	1	0.82	57	30	3.25	62.0	40.9	2.6	77.4	624	85.5
DOW02008018	WATTS BRANCH	4/17/2000	MT	68c	UPPER CUMBERLAND	2	2.2	46	25	3.14	85.0	66.7	1.8	75.5	732	90.5
DOW02008019	PUNCHEONCAMP BRANCH	4/18/2000	MT	68c	UPPER CUMBERLAND	2	1.7	55	30	2.89	82.3	70.2	2.7	70.1	785	93.5
DOW02008020	ROCK CREEK3 UT	4/18/2000	MT	68c	UPPER CUMBERLAND	2	0.63	56	26	2.68	74.9	52.1	2.0	82.4	666	89.2
DOW02008021	ROCK CREEK2 UT	4/18/2000	MT	68c	UPPER CUMBERLAND	1	0.37	39	19	2.47	81.8	70.7	0.9	75.6	352	87.1
DOW02008022	ROCK CREEK4 UT	4/18/2000	MT	68c	UPPER CUMBERLAND	2	0.89	37	21	2.98	86.5	73.4	3.0	51.8	623	82.6
DOW02023004	DRY FORK	4/19/2001	MT	68c	UPPER CUMBERLAND	2	2.05	34	18	3.67	34.5	25.9	0.5	68.8	6486	65.6
DOW02041003	BROWNIES CREEK1	4/26/2000	MT	69e	UPPER CUMBERLAND	2	2.3	52	31	2.93	50.1	18.4	2.2	35.8	495	71.1
DOW02041004	BROWNIES CREEK UT	4/26/2000	MT	69e	UPPER CUMBERLAND	1	0.31	39	24	2.53	36.3	18.2	0.7	29.5	1129	62.6
DOW02042003	WATTS CREEK	3/29/2001	MT	69e	UPPER CUMBERLAND	2	0.85	61	34	2.14	68.1	17.3	6.7	66.2	417	83.3
DOW02043006	ROUGH BRANCH UT	4/24/2002	MT	69e	UPPER CUMBERLAND	1	0.13	33	21	1.71	96.4	43.6	0.9	56.4	110	79.4
DOW02046002	BAD BRANCH	4/27/2000	MT	69e	UPPER CUMBERLAND	2	2.6	38	18	3.02	79.6	7.5	4.5	17.0	358	60.8
DOW02046004	PRESLEY HOUSE BRANCH	4/27/2000	MT	69e	UPPER CUMBERLAND	2	0.9	46	24	2.57	72.1	26.0	2.8	42.4	323	73.9
DOW04036017	STEER FORK	4/18/2001	MT	70f	KENTUCKY	2	3	59	36	3.03	84.8	62.1	4.7	78.1	1658	95.7
DOW04036022	HUGHES FORK	4/18/2001	MT	70f	KENTUCKY	1	1.35	64	34	2.75	58.5	33.7	9.9	61.2	1702	83.2
DOW04050002	CLEMONS FORK2	5/14/1999	MT	69d	KENTUCKY	2	2	66	32	3.11	59.8	35.8	13.0	54.9	408	81.1
DOW04050010	CLEMONS FORK3	4/10/2000	MT	69d	KENTUCKY	2	0.8	59	30	2.55	74.1	52.0	2.7	69.8	483	90.5
DOW04050011	FALLING ROCK BRANCH	4/11/2000	MT	69d	KENTUCKY	1	0.41	57	32	2.79	71.7	46.9	2.4	68.8	717	88.9
DOW04050012	JOHN CARPENTER FORK	4/12/2000	MT	69d	KENTUCKY	1	0.58	40	22	2.98	59.9	43.0	0.9	63.2	342	76.7
DOW04050013	SHELLY ROCK FORK	4/11/2000	MT	69d	KENTUCKY	1	0.55	38	20	2.41	78.8	62.1	0.7	73.3	430	85.6
DOW04050014	MILLSEAT BRANCH	4/11/2000	MT	69d	KENTUCKY	2	0.58	53	31	2.45	75.4	24.9	7.4	62.0	297	82.0
DOW04050015	LITTLE MILLSEAT BRANCH	4/12/2000	MT	69d	KENTUCKY	2	0.82	44	28	2.61	79.7	57.6	0.4	60.7	448	86.8
DOW04050019	ROARING FORK	4/23/2003	MT		KENTUCKY	1	0.38	49	28	1.91	86.6	51.5	7.0	55.8	344	86.8
DOW04052017	LITTLE DOUBLE CREEK	3/29/2000	MT	69d	KENTUCKY	2	1.5	27	19	2.16	94.3	64.1	0.0	50.1	749	80.4
DOW04052018	RIGHT FORK BIG DOUBLE CREEK2	3/29/2000	MT	69d	KENTUCKY	2	1.46	46	22	2.39	68.8	46.5	3.0	65.3	634	81.5
DOW04052019	LEFT FORK BIG DOUBLE CREEK	3/29/2000	MT	69d	KENTUCKY	2	0.6	52	25	2.55	74.4	54.1	1.5	70.6	782	87.6
DOW04052020	RIGHT FORK ELISHA CREEK	3/30/2000	MT	69d	KENTUCKY	2	2.35	48	31	2.63	72.0	48.0	4.5	54.3	690	83.9
DOW04052021	BIG MIDDLE FORK ELISHA CREEK	3/30/2000	MT	69d	KENTUCKY	1	0.82	57	28	2.82	74.4	55.9	5.5	41.7	542	83.9
DOW04052022	LEFT FORK ELISHA CREEK	3/30/2000	MT	69d	KENTUCKY	2	2.47	42	25	2.52	81.8	69.3	0.5	52.9	577	86.0
DOW04052023	RIGHT FORK BIG DOUBLE CREEK	4/5/2000	MT	69d	KENTUCKY	2	1.53	40	22	2.45	82.2	59.3	4.7	68.1	467	85.2
DOW04052030	SUGAR CREEK	4/6/2000	MT	69d	KENTUCKY	2	3.05	54	29	2.79	73.0	52.1	2.3	71.9	434	88.8
DOW04054005	CAWOOD BRANCH UT	3/28/2001	MT	69d	KENTUCKY	1	0.8	38	20	2.95	58.1	21.6	3.8	58.1	394	69.2
DOW04054009	BILL BRANCH	3/28/2001	MT	69d	KENTUCKY	2	2.3	43	28	1.99	91.2	59.2	2.0	83.0	294	91.5
DOW04054010	HONEY BRANCH	3/28/2001	MT	69d	KENTUCKY	2	0.82	40	26	2.83	86.4	65.3	2.3	81.7	427	90.0
DOW05037002	BOTTS FORK	4/18/2002	MT	70g	LICKING	3	3.38	55	31	3.31	63.9	37.3	13.9	61.1	1403	80.6
DOW05037004	WELCH FORK	4/18/2002	MT	70g	LICKING	2	1.5	62	36	2.62	67.5	28.8	8.0	62.9	375	84.2
DOW06012003	NICHOLS FORK	4/29/2002	MT	70f	LITTLE SANDY	2	0.65	49	25	2.95	73.8	31.5	4.0	43.9	321	75.8
DOW06012004	MEADOW BRANCH	4/30/2002	MT	70f	LITTLE SANDY	2	0.93	53	24	3.10	73.7	29.6	5.1	36.2	334	74.0
DOW06012009	GREEN BRANCH	4/29/2002	MT		LITTLE SANDY	2	1.17	49	22	3.42	63.8	14.7	7.2	42.3	265	67.6
DOW06013014	NEWCOMBE CREEK UT	3/14/2002	MT	70f	LITTLE SANDY	1	0.25	41	17	3.71	77.4	28.3	2.3	31.7	650	67.0

Appendix D. Metric and MBI values for Pennyroyal (PR) wadeable and headwater sites.

Wadeable

StationID	StreamName	CollDate	Bioregion	Sub-Ecoregion	Basin	Order	Drainage Area	G-TR	G-EPT	mHBI	m%EPT	%CO	%ClngP	TotInd	MBI
DOW02001003	MUD CAMP CREEK	6/14/00	PR	71h	UPPER CUMBERLAND	3	15.5	61	20	5.01	41.1	18.9	50.9	988	71.4
DOW02002002	HOWARDS CREEK	6/13/00	PR	71g	UPPER CUMBERLAND	3	11.2	67	29	4.24	43.1	21.8	51.5	864	79.7
DOW02002003	SULPHUR CREEK1	6/13/00	PR	71g	UPPER CUMBERLAND	3	5.2	61	23	4.23	52.5	11.7	42.7	634	77.0
DOW02003001	SPRING CREEK	7/28/00	PR	71g	UPPER CUMBERLAND	4	53.9	65	26	4.27	40.8	6.9	45.2	639	78.1
DOW02012001	BUCK CREEK	7/11/00	PR	71g	UPPER CUMBERLAND	5	172.2	80	31	4.32	36.7	0.7	56.8	1379	84.9
DOW02012001	BUCK CREEK	7/28/99	PR	71g	UPPER CUMBERLAND	5	172.2	62	26	4.18	56.8	0.9	65.9	449	87.0
DOW02012002	BRUSHY CREEK	7/11/00	PR	71g	UPPER CUMBERLAND	4	34.8	76	26	4.49	31.2	1.7	73.1	648	84.6
DOW02012002	BRUSHY CREEK	7/28/99	PR	71g	UPPER CUMBERLAND	4	34.8	60	23	3.96	55.7	6.7	59.6	433	82.7
DOW03008011	LINDERS CREEK	7/10/01	PR	71a	GREEN	3	26.2	61	23	4.38	42.2	2.9	66.6	1402	81.1
DOW03008016	MEETING CREEK	7/11/01	PR	71a	GREEN	4	26.1	65	21	4.47	26.6	11.7	30.8	1159	67.6
DOW03008020	ROUGH RIVER	7/10/01	PR	71a	GREEN	4	54.3	64	23	4.23	49.4	3.8	50.4	581	80.0
DOW03012008	ELK LICK CREEK	6/26/01	PR	71a	GREEN	4	22.9	70	25	4.98	61.5	11.0	72.2	2183	87.1
DOW03016002	BEAVERDAM CREEK1	6/28/01	PR	71a	GREEN	3	10.8	77	29	4.28	48.4	10.5	57.3	1062	85.6
DOW03016007	ALEXANDER CREEK	6/27/01	PR	71a	GREEN	3	4.77	66	24	4.55	41.9	10.5	50.6	1169	77.4
DOW03018011	GASPER RIVER	6/26/01	PR	71a	GREEN	3	26.3	50	17	4.75	36.6	4.1	72.4	1184	74.2
DOW03019016	TRAMMEL FORK1	7/19/01	PR	71e	GREEN	4	99.2	65	27	4.53	72.8	1.7	35.7	842	84.1
DOW03019017	TRAMMEL FORK2	7/20/01	PR	71g	GREEN	3	31.9	78	35	3.91	73.4	0.7	39.1	2297	90.2
DOW03019018	LICK CREEK	7/20/01	PR	71e	GREEN	3	12	64	23	4.40	25.8	3.1	87.6	2283	79.6
DOW03019025	W.F. DRAKES CREEK	7/19/01	PR	71e	GREEN	4	41.3	55	25	4.21	76.0	0.9	36.0	1526	81.8
DOW03021001	PETER CREEK	7/24/01	PR	71g	GREEN	4	60	59	22	4.79	78.2	1.6	49.9	611	82.6
DOW03021002	CANEY FORK	7/24/01	PR	71g	GREEN	3	11.4	61	24	4.61	53.3	2.0	67.3	1660	83.9
DOW03024019	LITTLE RUSSELL CREEK	8/14/01	PR	71g	GREEN	3	7.9	57	24	4.25	72.6	3.1	70.1	1781	88.8
DOW03024020	LYNN CAMP CREEK	7/21/01	PR	71g	GREEN	4	35.7	70	28	4.51	67.7	2.2	51.7	864	88.2
DOW03025004	CANE RUN	6/28/01	PR	71a	GREEN	3	8.5	69	27	4.18	50.6	4.4	69.4	2287	87.9
DOW03029005	E.F. LITTLE BARREN RIVER	8/14/01	PR	71g	GREEN	4	25	57	21	4.76	53.8	9.6	75.3	1267	81.4
DOW03030005	RUSSELL CREEK1	7/21/99	PR	71g	GREEN	4	189.1	65	26	4.46	41.1	2.1	68.9	470	83.9
DOW03030006	RUSSELL CREEK2	7/21/99	PR	71g	GREEN	3	16.5	59	21	4.62	53.0	3.5	45.0	706	76.4
DOW03030006	RUSSELL CREEK2	8/15/01	PR	71g	GREEN	3	16.5	77	24	4.83	55.3	17.0	40.2	749	78.1
DOW03031001	GOOSE CREEK	6/12/01	PR	71g	GREEN	4	40.1	60	25	4.71	55.1	4.4	49.9	735	80.1
DOW12035002	SALT LICK CREEK	6/24/99	PR	71c	SALT	4	5	66	31	4.35	50.6	3.4	53.8	409	85.1
DOW12035003	OTTER CREEK	6/24/99	PR	71c	SALT	4	14	56	21	4.60	60.2	5.5	45.7	532	77.3
DOW20005001	DONALDSON CREEK	6/19/01	PR	71f	LOWER CUMBERLAND	4	17.2	68	23	4.49	71.7	8.5	50.1	848	84.5
DOW20015001	WEST FORK RED RIVER	8/31/00	PR	71e	LOWER CUMBERLAND	4	178	55	15	4.82	48.3	2.3	56.2	1006	73.4
DOW20019004	ELK FORK	8/10/00	PR	71e	LOWER CUMBERLAND	4	88.5	43	17	4.02	53.8	0.7	58.7	866	75.8
DOW20020007	WHIPPOORWILL CREEK	8/10/00	PR	71e	LOWER CUMBERLAND	5	111	54	18	4.39	44.7	1.6	56.8	555	75.3

Headwater

StationID	StreamName	CollDate	Bioregion	Sub-Ecoregion	Basin	Order	Drainage Area	G-TR	G-EPT	mHBI	m%EPT	%Ephem	%C+O	%ClngP	TotInd	MBI
DOW10013001	PINEY CREEK	4/16/2002	PR	71a	TRADEWATER	3	3.86	44	16	3.49	83.2	24.1	1.7	68.3	870	74.7
DOW10013002	PINEY CREEK UT	4/16/2002	PR	71a	TRADEWATER	3	4.3	42	17	3.43	86.9	33.6	1.5	72.3	411	78.2
DOW10014005	SANDLICK CREEK UT	4/16/2002	PR	71a	TRADEWATER	1	0.95	32	18	3.52	76.1	32.3	0.9	47.0	347	69.5
DOW10014006	SANDLICK CREEK	4/16/2002	PR	71a	TRADEWATER	3	3.45	44	21	3.59	64.2	24.7	1.1	61.7	822	72.5
DOW12034003	OVERALLS CREEK	5/10/1999	PR	71c	SALT	2	2.4	42	22	3.45	81.5	39.8	7.0	48.9	601	75.6
DOW12034004	HARTS RUN	5/10/1999	PR	71c	SALT	2	2.25	35	21	3.29	72.9	38.6	1.2	31.2	414	69.7
DOW03016003	SULPHUR BRANCH	5/12/1999	PR	71a	GREEN	2	1.65	63	24	4.04	49.4	34.4	30.5	43.0	899	69.2
DOW03031011	GREEN RIVER UT	5/12/2003	PR	71g	GREEN	2	1.15	49	23	4.36	61.8	28.4	16.5	33.9	976	66.0
DOW03031013	ELLIS FORK	5/12/2003	PR	71g	GREEN	2	2.6	53	28	3.67	69.5	35.9	20.6	37.3	866	73.3
DOW03031012	WHITE OAK CREEK UT	5/13/2003	PR	71g	GREEN	2	2.17	57	28	3.74	68.2	20.7	12.1	43.2	801	73.0
DOW03007007	LITTLE SHORT CREEK	5/8/2002	PR	72h	GREEN	2	2	43	20	4.22	59.4	18.2	14.7	66.5	313	67.5
DOW03007008	POND RUN	5/8/2002	PR	72h	GREEN	3	4.53	52	28	4.04	75.6	36.9	7.4	71.7	336	82.0
DOW03007009	POND RUN UT	5/8/2002	PR	72h	GREEN	1	0.6	35	15	3.98	61.2	30.9	16.3	57.3	178	65.0
DOW03008014	NORTH FORK ROUGH RIVER	5/9/2001	PR	71a	GREEN	3	3.8	68	28	4.50	86.2	48.4	3.7	57.5	2041	85.7

Appendix E. Metric and MBI values for Miss. Valley-Int. River (MVIR) wadeable and headwater sites.

Wadeable

StationID	StreamName	CollDate	Bioregion	Sub-Ecoregion	Basin	Order	Catchment Area	G-TR	G-EPT	HBI2	m%EPT	%CO	%ClngP	TotInd	MBI
DOW03004002	MCFARLAND CREEK	6/20/01	MVIR	72c	GREEN	4	21.5	65	14	6.85	13.3	4.1	24.7	1101	54.8
DOW03004003	WEST FORK POND RIVER	6/20/01	MVIR	72h	GREEN	5	38.7	43	11	6.14	18.4	3.2	44.9	501	55.7
DOW03008017	CLIFTY CREEK1	7/11/01	MVIR	71a	GREEN	4	20.45	52	14	6.04	24.9	6.5	32.3	341	57.8
DOW03012009	CLIFTY CREEK2	6/27/01	MVIR	72h	GREEN	4	15.6	51	10	7.03	18.1	9.9	29.5	353	50.2
DOW03016005	BEAVERDAM CREEK2	7/25/01	MVIR	72h	GREEN	3	19.8	59	19	5.84	40.3	11.7	53.2	472	70.0
DOW03016006	ALEXANDER CREEK	7/25/01	MVIR	72h	GREEN	4	13.5	56	10	6.12	11.5	8.4	46.5	454	56.1
DOW07014006	OBION CREEK	5/19/00	MVIR	74b	MISSISSIPPI	5	185	52	13	6.51	24.2	8.3	14.4	1406	51.6
DOW07014006	OBION CREEK	6/ 8/00	MVIR	74b	MISSISSIPPI	5	185	47	12	6.18	14.3	24.1	31.7	328	49.7
DOW07023002	BAYOU DE CHIEN	5/10/00	MVIR	74b	MISSISSIPPI	4	48	70	15	6.87	21.4	13.4	16.0	583	54.7
DOW08007003	WEST FORK MASSAC CREEK	5/19/00	MVIR	74b	OHIO	4	18.8	37	6	6.54	33.5	15.3	26.2	275	47.8
DOW08007004	MASSAC CREEK2	5/19/00	MVIR	74b	OHIO	5	32	57	15	6.54	49.4	23.4	11.1	441	56.2
DOW08011001	COEFIELD CREEK	6/19/01	MVIR	71a	OHIO	4	17.6	50	10	6.50	9.3	4.9	19.1	614	47.7
DOW08032001	CLOVER CREEK	6/21/01	MVIR	71a	OHIO	4	24.03	54	11	6.49	8.5	12.1	42.5	819	53.1
DOW09010001	PANTHER CREEK1	5/18/00	MVIR	74b	TENNESSEE	4	20.9	64	14	6.22	40.8	25.7	29.7	377	59.8
DOW09010003	SOLDIER CREEK	6/ 8/00	MVIR	74b	TENNESSEE	4	14	48	14	6.31	13.6	18.9	39.8	264	53.2
DOW09010004	WEST FORK CLARKS RIVER	9/30/97	MVIR	74b	TENNESSEE	5	68	65	18	6.32	37.0	13.4	33.1	641	64.0
DOW09010004	WEST FORK CLARKS RIVER	8/16/00	MVIR	74b	TENNESSEE	5	68	64	22	6.32	44.6	13.9	33.3	648	67.7
DOW09016001	BLOOD RIVER	5/18/00	MVIR	71f	TENNESSEE	4	34.2	74	23	5.55	37.1	18.4	30.5	407	69.3
DOW09016002	PANTHER CREEK2	5/18/00	MVIR	71f	TENNESSEE	3	6.5	75	24	5.79	35.1	20.0	30.2	424	68.5
DOW10005005	HOODS CREEK	6/18/01	MVIR	72h	TRADEWATER	3	5.6	42	7	7.58	18.0	9.0	20.3	266	43.2
DOW20001001	SUGAR CREEK	6/ 7/00	MVIR	71f	LOWER CUMBERLAND	3	9	34	5	7.51	5.8	9.3	8.0	226	34.8
DOW20001002	CLAYLICK CREEK	6/ 9/00	MVIR	71a	LOWER CUMBERLAND	4	45	49	11	5.74	10.1	6.7	37.0	387	53.8
DOW20005004	CROOKED CREEK	6/ 7/00	MVIR	71f	LOWER CUMBERLAND	3	4.1	43	18	5.56	31.9	18.6	43.0	279	61.1

Headwater

StationID	StreamName	CollDate	Bioregion	Sub-Ecoregion	Basin	Order	Drainage Area	G-TR	G-EPT	HBI2	m%EPT	%Ephem	%C+O	%ClngP	TotInd	MBI
DOW03007006	HALLS CREEK	5/10/2002	MVIR	72h	GREEN	3	3.45	44	21	4.17	59.1	35.7	11.7	56.4	342	70.5
DOW03009002	MUDDY CREEK	5/9/2002	MVIR	72h	GREEN	2	1.9	51	17	6.06	45.9	38.9	16.0	27.5	244	59.3
DOW03013001	SIXES CREEK	5/9/2002	MVIR	72h	GREEN	3	3.8	42	19	5.12	67.5	27.6	3.3	62.6	246	69.5
DOW07023004	JACKSON CREEK	5/19/2000	MVIR	74b	MISSISSIPPI	3	2.6	52	15	6.79	23.6	17.2	24.4	16.8	250	45.8
DOW08007005	MASSAC CREEK UT	4/15/2002	MVIR	72a	OHIO	2	1	31	13	4.60	71.7	46.2	6.0	36.4	184	64.7
DOW09010010	PANTHER CREEK UT	4/29/2003	MVIR	74b	TENNESSEE	1	0.57	37	12	4.90	53.1	28.5	31.4	41.5	207	55.5
DOW09010011	HOMINY BRANCH	4/29/2003	MVIR	74b	TENNESSEE	2	0.48	27	11	4.68	77.9	36.3	10.7	37.0	281	61.1
DOW09016005	WILDCAT CREEK	4/29/2003	MVIR	74b	TENNESSEE	3	3.72	39	12	5.39	37.6	18.0	27.3	32.2	205	49.1
DOW09016008	SUGAR CREEK	4/2/2003	MVIR	71f	TENNESSEE	4	5.5	73	18	6.14	46.4	41.0	36.6	31.2	519	60.6
DOW09016010	GRINDSTONE CREEK	4/29/2003	MVIR	71f	TENNESSEE	2	1.35	39	14	4.55	53.1	38.8	22.4	25.7	245	57.9
DOW10011002	EAST FORK FLYNN FORK	4/15/2002	MVIR	72h	TRADEWATER	3	3.13	32	15	4.94	68.4	27.8	2.4	31.6	288	60.2
DOW20005006	FULTON CREEK UT	4/28/2003	MVIR	71f	LOWER CUMBERLAND	1	0.61	35	14	4.45	79.2	44.9	6.1	14.1	312	63.0
DOW20005007	FULTON CREEK UT	4/28/2003	MVIR	71f	LOWER CUMBERLAND	2	2.05	36	14	4.03	76.1	38.2	6.6	18.8	272	62.8